Titre: Title:	Chlorination of Toxic Cyanobacterial Cells and their Associated Toxins
Auteur: Author:	Yan Fan
Date:	2012
Type:	Mémoire ou thèse / Dissertation or Thesis
Référence: Citation:	Fan, Y. (2012). Chlorination of Toxic Cyanobacterial Cells and their Associated Toxins [Master's thesis, École Polytechnique de Montréal]. PolyPublie. https://publications.polymtl.ca/1060/

UNIVERSITÉ DE MONTRÉAL

CHLORINATION OF TOXIC CYANOBACTERIAL CELLS AND THEIR ASSOCIATED TOXINS

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MÉMOIRE PRÉSENTÉ EN VUE DE L'OBTENTION

DU DIPLÔME DE MAÎTRISE ÈS SCIENCES APPLIQUÉES

(GÉNIE CIVIL)

SEPTEMBRE 2012

UNIVERSITÉ DE MONTRÉAL

ÉCOLE POLYTECHNIQUE DE MONTRÉAL

Ce mémoire intitulé:

CHLORINATION OF TOXIC CYANOBACTERIAL CELLS AND THEIR ASSOCIATED TOXINS

présenté par : FAN Yan

en vue de l'obtention du diplôme de : Maîtrise ès sciences appliquées

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DEDICATION

To my family in China

For their unconditional love and support throughout my life

ACKNOWLEDGEMENTS

It is a pleasure to thank the many people who made this thesis possible.

Firstly, I would like to express my appreciation to my supervisors Dr. Michèle Prévost and Dr. Benoit Barbeau. Thanks for accepting me as a student and giving me the opportunity to be part of the CREDEAU. I would have been lost without their teaching, guidance, and patient encouragement.

I would also like to thank Dr. Arash Zamyadi for his time, his advices and his great efforts to explain details clearly and simply. It has been a great honour to work with him.

My gratitude also goes to the secretaries and the whole laboratory in the Department of CGM for helping me in many different ways. The assistance of Shokoufeh Nour, France Boisclair, Laura Razafinjanahary, Manon Latour, Jacinthe Mailly, Julie Philibert, Marcelin Fosting, Yves Fontaine, Mireille Blais, Sisi Zhao, Natasha McQuaid, and Yan Wang is gratefully acknowledged.

This study was financially supported by Natural Sciences and Engineering Research Council (NSERC) Canada and partners of Industrial Chair on Drinking Water Treatment at Ecole Polyechnique of Montreal.

RÉSUMÉ

Les cyanobactéries (algues bleu-verts), sont des microorganismes photosynthétiques aérobies qui sont présentes dans les milieux aquatiques situés partout sur le globe. L'eutrophisation croissante des eaux et le changement climatique tendent à favoriser la prolifération de cyanobactéries et la formation des fleurs d'eau (blooms). Les cyanobactéries sont généralement considérées comme des sources de goût et d'odeur dans les systèmes d'approvisionnement en eau potable. En particulier, les cyanotoxines produites par certaines espèces de cyanobactéries peuvent nuire à la santé humaine et animale. Par conséquent, les cyanobactéries suscitent une grande inquiétude dans le monde entier.

La pré-chloration est utilisée dans certaines usines de traitement des eaux en Amérique du Nord. La chloration directe de cyanobactéries peut permettre d'enlever des cellules et des toxines. Toutefois, elle peut aussi causer des problèmes de qualité de l'eau, telles que la lyse cellulaire, le relargage des toxines et du COD (Carbone organique dissous), l'augmentation de la demande en chlore et la formation des sous-produits de désinfection (SPD).

La présente étude vise à examiner les effets de la pré-chloration des cyanobactéries (*Microcystis aeruginosa* cultivée et fleurs d'eau naturels) et leur toxines associées. Les objectifs spécifiques de ce projet sont de : (1) déterminer et modéliser la lyse cellulaire de *M. aeruginosa,* le relargage des toxines et du COD et l'oxydation des MCs (Microcystines) pendant la chloration de l'eau ultra-pure et de l'eau naturelle dopée avec des cultures en laboratoire; (2) estimer la demande en chlore pour chaque cellule de *M. aeruginosa* lorsque la lyse cellulaire survient suite à la chloration; (3) étudier la formation de SPD associés avec la chloration des composés provenant de la cellule de *M. aeruginosa*; (4) évaluer l'efficacité de la chloration des cellules des cyanobactéries toxiques dans des échantillons naturels de fleurs d'eau toxiques.

Les valeurs de CT (l'exposition au chlore) de 110.7 mg.min/L (pour l'eau de Rivière Mille-Île dopée avec des *M. aeruginosa* cultivée) et 130.3 mg.min/L (pour l'eau extra-pure dopée avec des *M. aeruginosa* cultivée) sont requis pour obtenir 76% de la lyse cellulaire et à oxyder les toxines libérées jusqu'à un niveau inférieur à la valeur spécifiée de 1.0 µg/L de MC-LR dans les Directives de l'OMS (Organisation mondiale de la Santé). Dans l'eau ultra-pure, les taux d'oxydation des toxines étaient similaire ou plus rapide que les taux de lyse cellulaire. Une valeur constante de 5.6±0.2 $pgCl_2$/cellule est estimée comme la demande en chlore de chaque cellule de

M. aeruginosa lorsque la lyse cellulaire survient suite à la chloration. Il n'existe pas de formation de SPD significative dans la chloration de suspension lysée de *M. aeruginosa* (500,000 cellules/L) dans l'eau ultra-pure. Les fleurs d'eau naturelle sont plus résistantes à la chloration que la suspension cellulaire des cultures en laboratoire.

ABSTRACT

Cyanobacteria (Blue-green algae) are photosynthetic micro-organisms commonly existing in most aquatic systems. Because of climate change and increasing eutrophication of waters, the occurrence of algae blooms is becoming more frequent worldwide. Cyanobacteria are widely considered as sources of taste and odour in drinking water supplies. Cyanotoxins produced by a number of cyanobacteria species are a hazard for the health of human and animals. Therefore, cyanobacteria are a global concern.

Pre-chlorination is still practiced in many North American water treatment plants. Direct chlorination of cyanobacteria can increase the removal of their cells and toxins. However, it may also cause some water quality problems, such as cell lysis, which leads to toxin and DOC (Dissolved organic carbon) release, an increase in chlorine demand and disinfection by-products formation.

This study is to examine the effects of pre-chlorination on cyanobacteria (cultured *Microcystis aeruginosa* and natural bloom) and their associated toxins. The major objectives of this work are: (1) to determine and to model *M. aeruginosa* cell lysis, toxin and DOC release, and MCs oxidation during chlorination of ultrapure and natural waters spiked with laboratory cultures; (2) to estimate the *M. aeruginosa* cellular chlorine demand when cell lysis occurs due to chlorination; (3) to study the DBP formation associated with the chlorination of *M. aeruginosa* cell-bound materials; (4) to evaluate the efficiency of chlorination of toxic cyanobacterial cells in natural bloom samples.

Chlorine exposure (CT) values of 110.7 mg.min/L (for cultured *M. aeruginosa* spiked in Mille-Île River water) and 130.3 mg.min/L (for cultured *M. aeruginosa* spiked in ultrapure water) were required to achieve 76% cell lysis and oxidation of released cell-bound toxins at levels below the WHO (World Health Organazation) guideline value of 1.0 µg/L for MC-LR in drinking water. In ultrapure water, the rates of toxin oxidation were similar or faster than the rates of cell lysis. A constant value of 5.6±0.2 pgCl_2/cell is estimated as the *M. aeruginosa* cellular chlorine demand when cell lysis occurs due to chlorination. There is no significant DBP formation in chlorination of lysed *M. aeruginosa* suspension (500,000 cells/L) in ultrapure water. Natural blooms are more resistant than cell suspension of laboratory cultures.

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LIST OF ABBREVIATIONS

ADN	3-amino-9-methoxy-2, 6, 8-trimethyl-10-phenyldeca-4, 6-dienoic acid
ANTXs	Anatoxins
AOM	Algogenic organic matter
CB	Cyanobacteria
Cl_2	Chlorine
CYL	Cylindrospermopsin
DBP	Disinfection by-products
DOC	Dissolved organic carbon
ELISA	Enzyme-linked immunosorbent assays
EOM	Extracellular organic matter
GTXs	Gonyautoxins
HPLC	High performance liquid chromatography
IOM	Intracellular organic matter
LD50	Lethal dose 50
LPS	Lipopolysaccharides
MCs	Micorystins
MC-LR	Microcystin- leucine arginine
Mdha	N-methyldehydroalanine
MMPB	3-menthoxy-2-methyl- 4-phenylbutyric acid
MS	Mass spectrograph
NODs	Nodularins
NOM	Natural organic matter
PDA	Photo-diode array

PPIA	Protein phosphatise inhibition assays
TOC	Total organic carbon
WHO	World Health Organization

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INTRODUCTION

Background

Nowadays, because of climate change (especially global warming) and increasing eutrophication of waters, the occurrence of cyanobacteria blooms is becoming more and more frequent and has been highlighted as a great concern worldwide. Cyanobacteria, as known as blue-green algae, are oxygenic photosynthetic prokaryotes commonly existing in almost every conceivable freshwater, marine and terrestrial environment and also can be found in some extreme habitats (Chorus & Bartram, 1999). A number of them can produce lethal toxins (cyanotoxins) as well as taste and odour substances, which are counted as severe problems in drinking water resources (Miao & Tao, 2009).

Cyanotoxins have been responsible for death of animals and human illness in many countries. Microcystins (MCs), which were first extracted from the genera of cyanobacteria *Microcystis aeruginosa*, are hepatotoxins and the most frequently detected cyanotoxins in harmful cyanobacteria blooms from fresh water (Chorus & Bartram, 1999). Because of liver damage caused by microcystins, the symptoms of human illness are gastroenteritis and allergic or irritation reactions (Brookes et al., 2008; Merel, Clément, & Thomas, 2010). Furthermore, MCs have also been reported as potential tumour promoters in rats and mice (I. R. Falconer, 1991; Ho et al., 2006). Thus, chronic exposure to even low concentrations of microcystins can result in a serious health issue in humans (de Figueiredo, Azeiteiro, Esteves, Gonçalves, & Pereira, 2004). Among MCs, microcystin-LR (MC-LR) is the most frequent and toxic MC with an LD50 of 50 µg/kg of body weight (E. M. Rodriguez, Acero, Spoof, & Meriluoto, 2008; Westrick, Szlag, Southwell, & Sinclair, 2010). The World Health Organization (WHO) has set a provisional guideline value of 1.0 µg/L for MC-LR in drinking water (World Health Organization (WHO), 1998). This value has been adopted and used as a basis in many countries; Canadian Guideline for Drinking Water Quality for MC-LR is a proposed value of 1.5 µg/L which was established by Health Canada (Health Canada, 2007).

In Canada, cyanobacteria and their toxins, mianly microcystins, have been detected commonly in fresh water sources, especially during the seasons of summer and autumn (Jones, Gurney, & Rocan, 1998; Kotak & Zurawell, 2007; McQuaid, Zamyadi, Prévost, Bird, & Dorner, 2011). In

Quebec, 30.9% of raw water samples collected in the supply of drinking water plants using high risk sources dominated by potentially toxic cyanobacteria in 2004; this percentage increased to 82.6% in 2006 (Robert, 2008). In addition, the maximum concentration of cyanotoxins detected in these samples of raw water was 5.35 μg/L equ. MC-LR (Carrière, Prévost, Zamyadi, Chevalier, & Barbeau, 2010; Robert, 2008). Obviously, to minimize the threat of toxins, the treatment for cyanobacteria and their toxins is necessary.

To choose proper treatment of cyanobacteria and cyanotoxins, it is important to consider both cell-bound and dissolved components. In aquatic systems, most of cyanotoxins usually remain contained within intact cells (cell-bound toxins), and the remaining are extracellular dissolved toxins (Chorus & Bartram, 1999; Donati, Drikas, Hayes, & Newcombe, 1994). Conventional treatment processes (coagulation, flocculation, sedimentation and filtration) is effective for removal of cyanobacterial cells but inadequate for removing dissolved cyanotoxins (Chow, Drikas, House, Burch, & Velzeboer, 1999; Svrcek & Smith, 2004). Chemical oxidation with some common oxidants, such as chlorine, chloramines, ozone, chlorine dioxide and potassium permanganate, is a promising option as a safe barrier against cyanobacteria and their toxins (Hou, Hu, Jia, & Liu, 2008; E. M. Rodriguez, et al., 2008). Furthermore, the oxidation could be considered as a pre-treatment function or a disinfection stage.

There are increasing interests regarding the pre-treatment by oxidation, because of a wide variety of uses including: control of biological growth, tastes and odours and oxidizing organic matter to improve the coagulation/flocculation (Chorus & Bartram, 1999). Also, pre-chlorination at the raw water intake or intermediate chlorination ahead of the filters are still practiced in many water treatment plants. In drinking water pre-treatment, the addition of oxidants such as chlorine (Cl_2) to untreated water prior to coagulation has been shown to increase the removal of cyanobacteria cells and improve the following treatment processes (J. Plummer & Edzwald, 2002). However, it can also cause several water quality problems by decomposing of cells, e.g. increased chlorine demand, elevated chlorination by-products (DBP) formation, the release of DOC and cell-bound toxin and consequent increase in dissolved toxin levels (Daly, Ho, & Brookes, 2007). Therefore, pre-chlorination should be investigated to choose the suitable treatment conditions (i.e. dose, pH, contact time), which can be more effective on killing cyanobacteria cell, oxidizing released toxins to non toxic materials, controlling the DOC release and avoiding the DBP formation. Otherwise,

it would cause cell lysis and released toxins might end up in treatment station even in treated water.

Objectives

This study is to examine the effects of chlorine pre-treatment on cyanobacterial cells (cultured *Microcystis aeruginasa* and natural bloom) and their associated toxins. The specific objectives of this research are to:

1. Determine and to model the *M. aeruginosa* cells lysis, toxins and DOC release, and MCs oxidation during chlorination of ultrapure and natural water spiked with laboratory cultures;
2. Estimate the *M. aeruginosa* cellular chlorine demand when cell lysis occurs due to chlorination;
3. Study the DBP formation associated with the chlorination of *M. aeruginosa* cell-bound materials;
4. Evaluate the efficiency of chlorination of toxic cyanobacterial cells in natural bloom samples.

Structure of the thesis

Chapter 1 will present the literature review by providing an in-depth review of the current knowledge of cyanobacteria and their toxins, the measurement methods of cyanotoxins and the treatment by chlorination. Chapter 2 will provide the materials and methods used for the laboratory testing. The results and discussions will be then presented in Chapter 3. Finally, the conclusions and recommendations will be given at the end in the Conclusion chapter.

CHAPTER 1 LITERATURE REVIEW

1.1 Cyanobacteria

Cyanobacteria, also known as blue-green algae, are a large and heterogeneous group of asexual photosynthetic micro-organisms that possess the ability to synthesize chlorophyll *a* (Hou, et al., 2008). They commonly exist in a wide variety of freshwater, marine and terrestrial environments and also can be found in some extreme habitats, such as hypersaline, hot springs (at temperature up to 72 ^{o}C), and deserts (Chorus & Bartram, 1999).

Cyanobacteria had been classified with the algae because of their mechanism of photosynthesis; however, they are unicellular or multicelluar prokaryotes (i.e. whose cells lack a cell nucleus and have no membrane-bound sub-cellular organelles) with gram-negative cell walls (Hou, et al., 2008); whereas the cells of algae and plants are eukaryotic. They are classified within Eubacteria due to their cell structure and consist of more than 120 genera and 2000 species (Gerrath, Wehr, & Sheath, 2003). Most cyanobacteria can grow with light as an energy source through CO_2-dependent oxygen-evolving photosynthesis (Chorus & Bartram, 1999). The photosynthetic pigments of cyanobacteria, which contribute to the variety of cell colors from blue-green to violet-red, are chlorophyll *a* and accessory light-harvesting pigments such as phycocyanin, allophycocyanin and phycoerythrin (Chorus & Bartram, 1999). Many species of cyanobacteria have the ability of nitrogen-fixing (convert atmospheric N_2 to NH_3) which contribute greatly to the nitrogen economy of aquatic and terrestrial habitats (Fay, 1992; Oliver & Ganf, 2000; Svrcek & Smith, 2004).

Cyanobacteria have been found as fossils in early Archaean rocks of Western Australia dated 3.3-3.5 billion year ago (Hudnell, 2008; Sedmak & Kosi, 1998). Cyanobacteria are thought to be one of the pioneer oxygen-producing photosynthetic organisms on the early Earth and likely responsible for the conversion of the atmosphere from anaerobic to aerobic (Chorus & Bartram, 1999; Svrcek & Smith, 2004). Today, they still remain important as primary producers in marine environment.

1.2 Harmful effects and occurrence of cyanobacterial blooms and cyanotoxins

As known to all, cyanobacteria are integral parts of many ecosystems. However, there has been an increasing occurrence of cyanobacterial blooms and cyanotoxins (Paul, 2008; Svrcek & Smith, 2004). Cyanobacterial bloom, which is a rapid enlarge or dense accumulation of numerous buoyant cyanobacterial cells or colonies in an aquatic systems, occur when a combination of favourable conditions such as nutrient enrichment (particularly phosphorus and nitrogen), warm temperature (15 ^{o}C to 30 ^{o}C), neutral to alkaline pH (pH 6 to 9), and calm stable waters (W.W. Carmichael, 1994; Hitzfeld, Höger, & Dietrich, 2000; Newcombe, House, Ho, Baker, & Burch, 2010). Therefore, the increased eutrophication and climate change (especially global warming) caused by human actions and activities, are majorly responsible for the formation of bloom (Anderson, Glibert, & Burkholder, 2002; El-Shehawy, Gorokhova, Fernández-Piñas, & del Campo, 2012; Paerl & Paul, 2012).

Cyanobacterial blooms are undesirable. They hinder drinking, recreational and agricultural use of water bodies by producing offensive taste and odour compounds (such as geosmin and 2-methylisoborneol) and contributing to total organic carbon (TOC) and turbidity (Nguyen et al., 2005; Taylor et al., 2006). More importantly, 60 to 70% of observed cyanobacterial bloom includes toxin-producing strains of cyanobacteria (Fawell, Hart, James, & Parr, 1993); and the substaintial amounts of harmful cyanotoxins can be released into watershed during the death and lysis of cells in a cyanobacterial bloom (W.W. Carmichael, 1994; W. W. Carmichael, 2001; Svrcek & Smith, 2004). Cyanotoxins, as secondary metabolites produced by a small group of cyanobacteria mainly include *Anabaena*, *Aphanizomenon*, *Cylindrospermopsis*, *Lyngbya*, *Microcystis*, *Nodularia*, *Nostoc*, *Oscillatoria*, and *Planktothrix*. They are accountable for animal poisoning, human gastroenteritis, dermal contact irritations, allergic response and primary liver and kidney damage in humans (W. W. Carmichael, 2001; Codd, 2000; Duy, Lam, Shaw, & Connell, 2000; I.R. Falconer & Humpage, 2005). The main toxins are microcystins, anatoxins, cylindrospermopsins and saxitoxins (Duy, et al., 2000).

The harmful cyanobacterial events due to the presence of cyanobacterial blooms and cyanotoxins have been found worldwide since the first report of animal deaths after livestock ingested water during a cyanobacterial bloom in Australia in 1878 (Codd, Lindsay, Young, Morrison, &

Metcalf, 2005). In May 2007, a harmful cyanobacterial bloom event occurred in a large area of China's third largest freshwater lake-Taihu of Wuxi which threatened the supply of drinking water and fisheries-related food supplies for more than 8 million people (Cong, Shi-xin, Yi, & Fu-li, 2009; Liu, Chen, Li, Huang, & Shen, 2011). In Scotland, Ireland and New Zealand, dog deaths have occurred after eating fragments of anatoxin-a- producing cyanobacteria and drinking the water nearby (Codd, et al., 2005). A study has shown that anatoxin-a- and microcystin-containing cyanobacterial blooms have resulted in numerous cattle deaths in small lakes at alpine summer pastures in Switzerland (Mez et al., 1997). Although many reports of cyanobacteria poisonings involve the death of animals, some publications have proven that it could also be fatal to humans (Svrcek & Smith, 2004). In Brazil, 52 deaths of dialysis patients from water contaminated with cyanotoxins were reported (Jochimsen et al., 1998).

Cyanobacteria associated with the production of microcystins were detected in 539 of 677 (80%) raw water sources in 45 North American cities (W.W. Carmichael, 2000). Another study showed that the microcystins have been found in the majority of raw water samples from 33 American water supplies; but only 7% of samples had more than 1 μg/L of microcystin (Haddix, Hughley, & Lechevallier, 2007). In Canada, cyanobacteria and their toxins, especially microcystins, have been detected in multiple lakes, some of which are drinking water sources (Jones, et al., 1998). In Alberta, cyanotoxins have been found in 67% of samples of raw water from drinking water facilities, with a maximum total microcystin concentration of 14.8 μg/L (Kotak & Zurawell, 2007; Zurawell, 2002). In Québec, 78.2% of raw water samples collected in the supply of plants using high risk sources contained one or more species of toxic cyanobacteria in 2004; this percentage increased to 87% in 2006. In addition, the maximum concentration of microcystins (in all forms) measured in raw water was 5.35 μg/L (Carrière, et al., 2010; Robert, 2008).

1.3 Classification of toxins and principal toxins

At least a third of the 50 known genera of cyanobacteria can produce toxins as secondary metabolites (Yoo, Carmichael, Hoehn, & Hrudey, 1995). The toxins are generated in all cyanobacteria growth cycle and remain mostly in the cell (cell-bound toxin) until age or stress result in their release into the surrounding water to form dissolved toxin during cell lysis (Svrcek & Smith, 2004). Cyanotoxins usually fall into three groups based on their chemical structure: cyclic peptides, alkaloids, and lipopolysaccharides (LPS) (Chorus & Bartram, 1999). They can

also be classified into four classes according to the mechanisms of toxicity: hepatotoxins, neurotoxins, general cytoxins, and endotoxins (Newcombe, Cook, Brooke, Ho, & Slyman, 2003; Yoo, et al., 1995). The cyanotoxins produced by the specific species of cyanobacteria and their health effects are listed in Appendix 1.

Hepatotoxins

Hepatotoxins are natural products with molecular weight of 800-1,100 Daltons that demage the liver. They contain either five (nodularins) or seven (microcystins) amino acids, with two terminal amino acids being joined to form cyclic compounds. The most frequently detected cyanotoxins in harmful cyanobacteria blooms from fresh water is the microcystin family (Brookes, et al., 2008; Chorus & Bartram, 1999).

Microcystin

Figure 1-1 shows the chemical structures of different variants of microcystins. Structurally, microcystins (MCs) are monocyclic heptapeptide made from seven amino acids with medium molecular masses around 1,000 Daltons (Westrick, et al., 2010). The conserved toxic moiety is 3-amino-9-methoxy-2, 6, 8-trimethyl-10-phenyldeca-4, 6-dienoic acid (Adda) and the other mostly conserved unit is N-methyldehydroalanine (Mdha) (Chorus & Bartram, 1999; Westrick, et al., 2010). They consist of more than 60 reported variants; however, microcystin-LR (MC-LR) with the variable amino acids leucine (L) and arginine (R) is the most frequent and toxic MC, with an LD_{50} value of 50 µg/kg (E. M. Rodriguez, et al., 2008; Westrick, et al., 2010). MC-RR, MC-YR and MC-LA also occur more frequently in freshwaters (de Figueiredo, et al., 2004). Based on animals studies of MC-LR toxicity, the World Health Organization (WHO) has set a provisional guideline value of 1.0 µg/L for MC-LR in drinking water (World Health Organization (WHO), 1998). This value has been adopted and used as a basis in many countries such as Brazil, New Zealand, and UK. Canadian Guideline for Drinking Water Quality for MC-LR is at the approval stage with a proposed value of 1.5 µg/L which was established by Health Canada (Health Canada, 2007). The Institut national de Santé publique du Québec also suggested a provisional value of 3.7 µg/L for anatoxin-a currently ((INSPQ), 2004).

Microcystins were first extracted from the genera of cyanobacteria *Microcystis aeruginosa* and have been found frequently in genera *Anabaena, Anabaenopsis, Aphanicapsa*, *Hapalosiphon,*

Microcystis, *Nastoc*, and *Oscillatoria* (Chorus & Bartram, 1999). Once absorbed by the organism, microcystins primary target is liver where protein phosphatase is inhibited and phosphorylated proteins are accumulated; they result in the disruption of the hepatocyte skeletal structure and cell integrity leading to acute or chronic liver injury (Brookes, et al., 2008; Merel, Clément, Mourot, Fessard, & Thomas, 2010). The symptoms of human illness attributed to the microcystins are gastroenteritis and allergic or irritation reactions (Brookes, et al., 2008). In addition, microcystins have also been reported as potential tumor promoters in rats and mice (I. R. Falconer, 1991; Ho, et al., 2006). Thus, chronic exposure to even low concentrations of microcystins can result in the promotion of cancer in humans (de Figueiredo, et al., 2004).

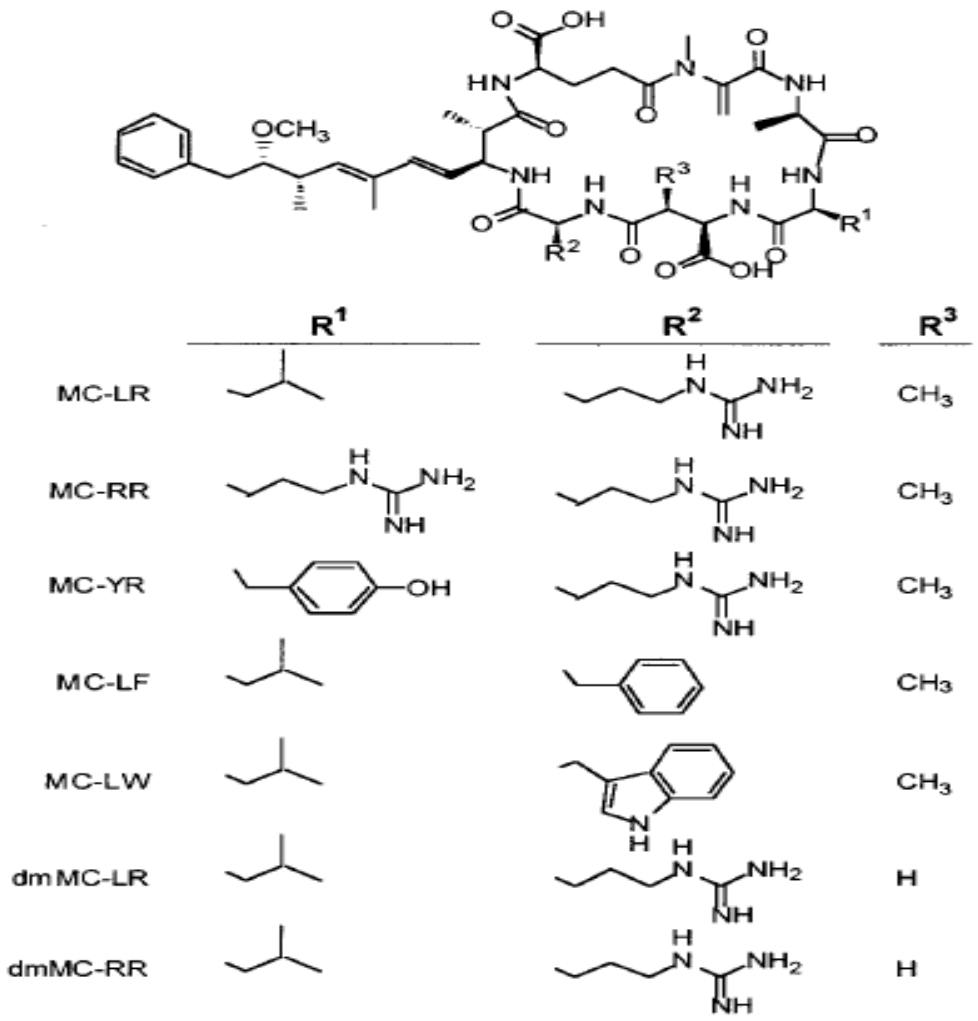

Figure 1-1: Chemical structures of different variants of microcystin (MC), 3-desmethylmicrocystins (dmMC) (from (Fischer et al., 2001))

1.4 Measurement methods for cyanotoxins

In order to monitor cyanotoxins in water systems and assess their behavior during water treatment, some robust and sensitive analytical measurement methods are required and have been developed (B. Nicholson & Shaw, 2011). The common analytical methods available for cyanotoxin range from immunological or biochemical screening techniques based on enzyme-linked immunosorbent assays (ELISA) and enzyme activity (protein phosphatase inhibition assays, PPIA) respectively, to quantitative chromatographic techniques based on high performance liquid chromatography (HPLC) with various detections (ultraviolet, UV; photo-diode array, PDA; mass spectrograph, MS or fluorescence detection) and more sophisticated liquid chromatography-mass spectrometry (LC-MS) (Newcombe, et al., 2010; B. C. Nicholson & Burch, 2001).

Capillary electrophoresis (CE) and 3-menthoxy-2-methyl-4-phenylbutyric acid (MMPB) method are less common analytical techniques and have been evaluated in some laboratories (B. Nicholson & Shaw, 2011; B. C. Nicholson & Burch, 2001). Animal bioassays (mouse tests) are also available for screening all toxic substances of cyanobacteria. A table comparing common methods of measuring cyanotoxins and general considerations for optimum measurement is presented in Appendix 2.

1.5 Treatment methods of cyanobacteria

As the final option to minimize the threat of cyanobacteria and cyanotoxins in water supplies, a number of physical, chemical and biological treatment technologies have been investigated in many reports. As mentioned previously, a normal cyanobacteria cell can contain high concentrations of toxin and/or taste and odour compounds confined within its wall (Chorus & Bartram, 1999; Newcombe, et al., 2010). In addition, cyanotoxins can be either found inside of cells (called intracellular/cell-bound cyanotoxins which can be released by cell lysis) or outside of cells (called extracellular/dissolved cyanotoxins) (Westrick, 2008). Especially under bloom conditions, a substantial proportion of cell-bound toxins would be released to the water column (Chorus & Bartram, 1999). Therefore, the efficiency of a treatment method depends on the form of the cyanobacteria (i.e. individual cells, filamentous etc), the total concentration of toxins, and whether it is dissolved or cell-bound. It is also important to consider whether the method causes

lysis of cells and oxidize dissolved toxins (Donati, et al., 1994; Hart, Fawell, & Croll, 1998; Svrcek & Smith, 2004).

Table 1.1 summarizes the water treatment techniques for cyanobacteria and their toxins.

Table 1.1: Techniques for treatment of cyanobacterial cells and their toxins (adapted from (Brookes, et al., 2008; The Cooperative Research Centre for Water Quality and Treatment (Australia), 2007; Arasah Zamyadi, 2011)

Treatment Process	Cell/toxins	Treatment Efficiency
Coagulation Sedimentation	Intact cells	Very effective for the removal of intracellular toxins; provided cells accumulated in sludge are isolated from the plant.
Rapid filtration	Intact cells	Very effective for the removal of intracellular toxins; provided cells are not allowed to accumulate on filter for prolonged periods.
Slow sand filtration	Intact cells	As for rapid sand filtration, with additional possibility of biological degradation of dissolved toxins.
Combined coagulation/ decantation/ filtration	Intact cells	Extremely effective for the removal of intracellular toxins; provided cells accumulated in sludge are isolated from the plant cells and any free cells are not allowed to accumulate on filter for prolonged periods.
Membrane processes	Intact cells	Very effective for the removal of intracellular toxins; provided cells are not allowed to accumulate on membrane for prolonged periods.
DAF	Intact cells	As for coagulation/sedimentation.
Oxidation processes	Intact cells	Not recommended as a treatment for cyanobacterial cells as this process can lead to cell damage and lysis and consequent increase in dissolved toxin levels.
Ozonation	All toxins	Ozonation is effective for all dissolved toxins except the saxitoxins. A residual of at least 0.3 mg/l for 5 minutes will be sufficient. Doses will depend on water quality.
Chlorination	All toxins	If a dose of at least 3 mg/l is applied and a residual of 0.5 mg/l is maintained for at least 30 minutes, most MCs and CYN should be destroyed. MC-LA and saxitoxins may require a higher residual. Limited data suggest chlorination is only effective at elevated pH for saxitoxins.
Chloramination	All toxins	Ineffective.
Chlorine dioxide	All toxins	Not effective with doses used in drinking water treatment.
Potassium permanganate	All toxins	Effective for MC, anatoxins and CYN, no data for saxitoxins.
Hydrogen peroxide	All toxins	Not effective on its own.
UV Radiation	All toxins	Capable of degrading MC-LR and CYN, but only at impractically high doses or in the presence of a catalyst.
Adsorption – PAC (doses required vary with water quality)	MCs (except MC-LA)	Wood-based, chemically activated carbon is the most effective, or coal-based carbon with similar pore distribution, 60 minutes contact time recommended.
	MC-LA	High doses required.
	CYN	Wood-based, chemically activated carbon is the most effective, or similar, 60 minutes contact time recommended.
	Saxitoxins	A microporous carbon (steam activated wood, coconut or coal based) 60 minutes contact time recommended effective for the most toxic of the variants.
Adsorption –GAC	All toxins	GAC adsorption displays a limited lifetime for all toxins. This can vary between 2 months to more than one year depending on the type of toxin and the water quality.
Biological filtration	All toxins	When functioning at the optimum this process can be very effective for the removal of most toxins. However, factors affecting the removal such as biofilm mass and composition, acclimation periods, temperature and water quality cannot be easily controlled.
Membrane Processes	All toxins	Depends on membrane pore size distribution.

Note: DAF, dissolved air flotation; PAC, powdered activated carbon; GAC, granular activated carbon; UV, ultraviolet.

1.5.1 Conventional treatment

Conventional treatment for the removal of cyanobacteria and cyanotoxins consist of coagulation, flocculation, separation (sedimentation/flotation), and filtration processes (Svrcek & Smith, 2004). Many studies have reported that the application of conventional treatment is effective for the removal of cyanobacterial cells but ineffective for the removal of dissolved cyanotoxins (Chorus & Bartram, 1999; Chow, et al., 1999; Himberg, Keijola, Hiisvirta, Pyysalo, & Sivonen, 1989; Svrcek & Smith, 2004).

The specific performances of unit process of conventional treatment are shown in Appendix 3.

1.5.2 Chemical oxidation / Chlorination

Chemical oxidation processes in drinking water treatment can control colour and odour, reduce COD/BOD, remove both organic and oxdisable inorganic components, enhance coagulation/flocculation and iron or manganese oxidation (Chorus & Bartram, 1999). Many studies have reported that chemical oxidation is an effective method and a safe barrier against cyanobacteria and their toxins in order to decrease public health risk (Miao & Tao, 2009; E. Rodriguez, Majado, Meriluoto, & Acero, 2007). The oxidants investigated most commonly for cyanobacteria and cyanotoxin treatment are chlorine, chlorine dioxide, chloramine, ozone, and potassium permanganate (E. Rodriguez, Majado, et al., 2007). Meanwhile, the removal effects of these oxidants could be examined in post-oxidation and also in pre-oxidation (before cell removal).

High concentration cyanobacteria blooms in drinking water sources can cause serious problems in various stages of conventional water treatment, including poor coagulation/flocculation and separation, filter clogging, increased disinfectants demand, and unpleasant odors and tastes (Yun, Lim, Cho, & Park, 1997). Pre-oxidation is used for untreated water to reduce the biological growth of cyanobacteria in treatment plant and improve coagulation/flocculation and filtration processes (Chorus & Bartram, 1999; Petrusevski, van Breeman, & Alaerts, 1995). Nevertheless, it may also cause cell death or lysis to release toxins and odour/taste compounds. Therefore, in the presence of cyanobacteria cells, the dosage of oxidant in pre-treatment should be sufficient to meet the demand of the water including cells, and a residual required for oxidize both cell-bound

and dissolved toxins to non toxic materials (Chorus & Bartram, 1999; Miao & Tao, 2009; Newcombe, et al., 2010).

Chlorination

Chlorine is the chemical oxidant most frequently used for disinfection in water treatment in North America (Svrcek & Smith, 2004). The most reactive form of chlorine is hypochlorous acid (HOCl) which is in equilibrium with hypochlorite ion (OCl^-) in solution. The chemical equation is given below:

$$HOCl <=> H^+ + OCl^-$$

The concentration of hypochlorous acid is dependent on the pH of water (Newcombe, et al., 2010). Table 1.2 shows the relative concentrations of the two major forms of chlorine over a moderate range of pH.

Table 1.2: Ratio of HOCl to OCl^- and concentrations of the species at different pH. Initial concentration 5.4 mg/L as Cl_2 (from (Newcombe, et al., 2010))

pH	**6.0**	**6.5**	**7.0**	**7.5**	**8.0**	**8.5**	**9.0**
HOCl : OCl^-	32 :1	10 :1	3.2 :1	1 :1	0.32 :1	0.1 :1	0.03 :1
HOCl (mg/L)	3.9	3.6	2.9	2.0	1.1	0.4	0.1
OCl^-(mg/L)	0.1	0.4	1.1	2.0	2.9	3.6	3.9

Early studies reported that chlorine was ineffective to destroy the microcystins, even at a substantial dosage of 5 mg/L (Himberg, et al., 1989). In these studies, chlorine may have been consumed by organic matter reported to be present, leaving no chlorine residual for the removal of microcystins; or chlorination may have carried out at a pH of relative low concentration of hypochlorous acid (Chorus & Bartram, 1999). Contrarily, some other studies demonstrated that chlorine has a potential capacity to remove cyanobacteria cells and some cyanotoxins under proper treatment conditions (Acero, Rodriguez, & Meriluoto, 2005; B. C. Nicholson, Rositano, & Burch, 1994; Tsuji et al., 1997). The post-oxidation during treatment, most often chlorination, is an essential barrier against dissolved and cell-bound cyanotoxins to prevent cells and toxins breakthrough (Pietsch, Bornmann, & Schmidt, 2002; A. Zamyadi et al., 2012). Direct chlorination of raw water or inter-chlorination is still very common in many North American water treatment plants. As known, there are many concerns regarding the pre-chlorination, especially in water containing significant cyanobacteria bloom, such as: cell damage and the

subsequent release of cell-bound cyanotoxins, increased chlorine demand, dissolved organic carbon (DOC) release and elevated harmful chlorination by-products (DBP) formation (Merel, Clément, Mourot, et al., 2010). However, it also may constitute a practical temporary treatment choice if it is proven to be efficient against both cell-bound and dissolved toxins, while still acceptable at short term in terms of other water quality parameters. Thus, it is very necessary to understand the conditions or limitations of post- and pre-chlorination of cyanobacteria cells and their toxins as used during drinking water treatment plant (DWTP) operation.

A study conducted by Nicholson et al. demonstrated that chlorine could be very effective to oxidize both microcystin and nodularin if a free chlorine residual of 0.5 mg/L were maintained after 30 min of contact time with pH value under 8 (B. C. Nicholson, et al., 1994). Other studies also showed that the efficiency of chlorination with cyanobacteria and their toxins depends on various experimental conditions (Merel, Clément, Mourot, et al., 2010; Newcombe & Nicholson, 2004; Xagoraraki et al., 2006).

For an efficient chlorination, the key parameters are the concentration of residual chlorine and the contact time. The appropriate degree of chlorination is estimated using the concept of concentration multiplied by time, chlorine exposure, or CT (Carrière, et al., 2010). The CT value is calculated by determining the area under a graph of chlorine concentration versus time (Ho, et al., 2006). The rates of cell lysis, MCs release, and MCs oxidation can present the degree of cell-bound and dissolved MCs chlorination under given water quality conditions (Daly, et al., 2007).

The effect of pH on chlorination of cyanotoxins, especially for MCs, was commonly investigated, and a correlation on destruction efficiency was observed in many researches (Acero, et al., 2005; B. C. Nicholson, et al., 1994; E. Rodriguez et al., 2007; E. Rodriguez, Sordo, Metcalf, & Acero, 2007; Svrcek & Smith, 2004). The reaction between microcystins and HOCl mainly occurred in the range of pH 6 to 9; the reduction of toxin removal was examined with increasing pH above 8 because of the decreasing concentration of HOCl (Svrcek & Smith, 2004). Xagoraraki, et al., 2006 (Xagoraraki, et al., 2006) showed that microcystin-LR inactivation rate constant (*k*) by free chlorine was estimated to be the highest at 0.065±0.007 L/ (mg min) at pH 6 and the lowest at 0.005±0.001 L/ (mg min) pH 9 at 11 °C. Acero et al., 2005 (Acero, et al., 2005) also determined that the apparent second-order rate constant for the chlorination of microcystin-LR varied from 475 $M^{-1}s^{-1}$ at pH 4.8 to 9.8 $M^{-1}s^{-1}$ at pH 8.8. Therefore, chlorine is very efficient for oxidizing

dissolved MCs under various water quality conditions and the degradation of MCs is favoured at low pHs (Chorus & Bartram, 1999). For other cyanotoxins, cylindrospermopsin (CYN) degradation via chlorination with a free chlorine residual of 0.5 mg/L within a pH 6 to 9 could achieve > 99% (Senogles et al., 2000). A study found that the maximum apparent second-order rate constant for the reactivity of CYN with chlorine was 1265 $M^{-1}s^{-1}$ at pH 7 (E. Rodriguez, Onstad, et al., 2007). Saxitoxins (STX) are not as reactive with chlorine as microcystins (Newcombe, et al., 2010). A significant removal of STX by chlorine was shown only at high pH 8 to 9; STX degradation augmented from 20% to 98% when pH increased form 4 to 9 with a free chlorine residual of 0.5 mg/L after 30 min of contact time (Newcombe, et al., 2010; B. C. Nicholson et al., 2003).

Chlorination was also found to be efficient to oxidize *M. aeruginosa* cells and subsequently cell-bound MCs using elevated concentrations of laboratory cultured cells (3×10^5–1×10^6 cells/mL) in natural water (Daly, et al., 2007; Merel, Clément, Mourot, et al., 2010). In a recent study of Lin et al., 2009 (Lin et al., 2009), results of cell integrity of high concentration have shown that laboratory grown *M.aeruginosa* in nature water may be more resistant to cell demage by chlorination than *Anabaena circinalis* and the small *M.aeruginosa* colonies from natural blooms were more resistant than cell suspension of laboratory cultures. Finally, there is very limited information about the chlorination of the low concentration of dissolved and cell-bound MC-LReq from a natural bloom.

The predictable rapid cell rupture and subsequent release and oxidation of both dissolved and cell-bound cyanotoxins can be described by first-order apparent rate constant in most laboratory testing. A study showed that the rate constants of MCs oxidation calculated from spiked dissolved toxins in Myponga Reservoir Water are 2 to 24 times higher than those estimated from the chlorination of released cell-bound toxins from cell suspension (Daly, et al., 2007). More modest differences (1.2-1.5×) constant rates have been reported using lower cell densities of *Anabaena circinalis* for cell bound saxitoxin and of *M. aeruginosa* for cell-bound MCs (A. Zamyadi, Ho, Newcombe, Bustamante, & Prévost, 2012; A. Zamyadi et al., 2010). Due to limited study showing higher cyanobacteria cell resistance and poor oxidation of dissolved toxins in the presence of cell rupture, the efficiency of the oxidation of released cell-bound toxins from natural blooms has been more frently concerned (A. Zamyadi, MacLeod, et al., 2012). Besides, the knowledge of *M. aeruginosa* cellular chlorine demand which is required for optimal operation

of chlorination in DWTP is still undetermined. This information is important to ensure that sufficient chlorine capacity is present during peak cell concentration events.

The chlorination of drinking water can produce trihalomethanes (THMs) and other chlorinated by-products. Cyanobacteria cell and their excreted metabolic products may potentially contribute to the DBP formation (Bond, Henriet, Goslan, Parsons, & Jefferson, 2009; Huang et al., 2009; J. D. Plummer & Edzwald, 2001). Release of cell-bound compounds due to autolysis of cyanobacteria cells is classified as extracellular organic matter (EOM) and due to compromised lysis is categorised as internal organic matter (IOM) (Huang, et al., 2009). Both EOM and IOM could promote THMs and haloacetic acids (HAAs) production, due to the chlorination of released compouds (Bond, et al., 2009; Huang, et al., 2009). Some studies have reported the significant DBP formation (e.g. total THM yields from 67 to 269 µg/L) in connection to reactive carbon associated with high cell numbers and EOM (Huang, et al., 2009; A. Zamyadi, Ho, et al., 2012). However, chlorination of approximately 46,000 cells/mL of *Anabaena circinalis* in natural water (complete cell damage and below detection limit oxidation of produced saxitoxins) with a CT of 50.3 mg.min/L only increased 9 µg/L of total THM (TTHM) and 3 µg/L of HAA9 compared to the control assay (chlorination of natural water without cyanobacteria cells)(A. Zamyadi, et al., 2010).

The DBP formation potentials of IOM and EOM in chlorination depend on the various conditions especially chlorine doses, growth phase, species and culture conditions (Huang, et al., 2009).

CHAPTER 2 MATERIALS AND METHODS

2.1 Materials / Chemicals and Reagents

A toxic strain of *M. aeruginosa*, isolated from Pretzlaff Pond, Alberta, Canada, was cultured in the laboratory in artificial seawater medium (ASM-1) according to the method of Gorham, McLachlan, Hammer, & Kim, 1964 (Gorham, McLachlan, Hammer, & Kim, 1964). Distilled water (20 L) was fortified with nutrients according to the ASM-1 preparation, sterilized at 120^{o}C, and then pH adjusted using NaOH 1M solution, inoculated with an ASM-1 grown culture in stationary phase. The culture was incubated at 26^{o}C under 6 hours rotating light-darkness flux at light intensity of 70 µmol s^{-1} m^{-2}. All chemicals and reagents used were of the purest available quality to analytical grade in laboratory from various suppliers. Solutions of chlorine, phosphate buffers, quench solution and analytical reagents were prepared with ultrapure water.

2.2 Water Source

Natural water and ultrapure water were both used for chlorination experiments with the laboratory cultured *M. aeruginosa*. The experimental plan also included chlorination of Missisquoi Bay bloom water samples prior to any treatment (without filtration).

MIR Water: Natural water was sourced from Mille-îles River (MIR) in Laval, Quebec, Canada. Samples were taken from the inlet of St-Rose drinking water treatment plant (DWTP) in Laval prior to any treatment on June 26, 2009. Untreated MIR water was filtered over 0.45 µm filters (HA 14250, Millipore) to remove suspended materials and then stored at 4 ^{o}C. The water had a dissolved organic carbon (DOC) of 6.3 mg/L as C, N-NH_3 of 112.4 µg/L as N, true color of 43 CU (Color Units), and UV absorbance at 254 nm of 0.191 cm^{-1}, pH of 7.4 and an alkalinity of 19 mg/L as $CaCO_3$. True color and UV were measured on spectrophotometer (Milton Roy 1001 Plus). Before the chlorination experiments, the temperature of filtered MIR water should be adjusted from 4 ^{o}C to 20±2 ^{o}C (ambient temperature).

Ultrapure water: Ultrapure Milli-Q water (Millipore Pty Ltd, USA, 18.2 MΩ.cm @25 ^{o}C, TOC of 1 µg/L) was used for selected experiments.

Missisquoi Bay bloom water samples: Environmental cyanobacterial bloom samples were collected from a bloom event in Missisquoi Bay of Lake Champlain in southern Quebec, in close

proximity of the water intake. In this water, the dissolved organic carbon (DOC) was 5.4 mg/L as C and the ambient pH was 7.8. The samples at cell number of approximately 5.7×10^5 (±4%) cells/mL incorporated seven different cyanobacteria species dominated by *Microcystis* sp (A. Zamyadi, MacLeod, et al., 2012).

Before all DOC analyses, samples were filtered through pre-rinsed (1 L ultrapure water) 0.45 µm cellulose nitrate Supor®-450 membrane filters (PALL Life Sciences, USA). DOC measurements were measured using a 5310C total organic carbon analyzer (Sievers Instruments Inc., USA). pH values were measured using a Fisher Scientific pH meter (Accumet, Fisher Scientific Instruments, USA) which was calibrated with pH 4, 7 and 10 standard buffers (Fisher, USA).

2.3 Chlorination experiments

In this study, the chlorination experiments were conducted in different series with various experimental conditions. Table 2.1 shows the summary of various chlorination experiments conducted in this study. The first set of chlorination experiments (1^{st} to 3^{rd} Cl_2 Exp) were conducted using cultured *M. aeruginosa*. The second set of chlorination experiments were conducted directly on (4^{th} Cl_2 Exp) natural cyanobacteria bloom samples from Missisquoi Bay.

Sous-section additionnelle.

Table 2.1: The summary of various chlorination experiments conducted in this study

Experiment No.	Cyanobacteria Source	Water Source	Water Quality	pH	Cl_2 Dose (mg/L)	Cyanobacteria Concentration (cells/mL)	Contact Time (min)	Measurement Parameters
1st Cl_2 Exp	Cultured *M.aeruginosa* in laboratory	Ultrapure water	25 ℃, TOC -1 µg/L	8	2	60,000 (±2%)	0, 1, 2, 5, 10, 15, 30, 60	Cl_2 Residual & Cl_2Demand; DOC; Extracellular MCs & Total MCs; Cell lysis
					3	250,000 (±4%)		
					4.5	500,000 (±4%)		
2nd Cl_2 Exp		MIR water from inlet of St-Rose DWTP	After filtration (0.45 µm): DOC - 6.38 mg/L as C, N-NH_3 -112.4 µg/L as N, True Color – 43 CU, UV absorbance (at 254 nm) - 0.191 cm^{-1}, pH - 7.4, Alkalinity - 19 mg/L as $CaCO_3$	7	2	50,000 (±2%)	0, 1, 2, 5, 10, 15, 30, 60	Cl_2 Residual & Cl_2 Demand; DOC; Extracellular MCs & Total MCs; Cell lysis
					5			
					10			
				8.5	2			
					5			
					10			
3rd Cl_2 Exp		Ultrapure water	25 ℃, TOC -1 µg/L	8	5.5	500,000 (±2%)	30, 1440	Cl_2 Residual & Cl_2 Demand; TTHM & HAA6
4th Cl_2 Exp	Environmental cyanobacterial bloom samples from Missisquoi Bay (Seven cyanobacteria species dominated by *Microcystis* sp)		DOC - 5.4 mg/L as C, pH -7.8	7.8	2	5.7×10^5 (±4%)	0, 1, 2, 5, 10, 15, 30, 60	Cl_2 Residual & Cl_2 Demand; DOC; Extracellular MCs & Total MCs; Cell lysis
					5			

Chlorine stock concentrations and free chlorine residuals were determined using the DPD-FAS titration method (4500Cl-F. DPD Ferrous Titrimetric Method) described in Standard Methods (American Public Health Association (APHA), American Water Works Association (AWWA), & Water Environment Federation (WEF), 2005). Four chlorination experiments were conducted separately on: (1^{st} Cl_2 Exp) *M. aeruginosa* suspensions in ultrapure water; (2^{nd} Cl_2 Exp) *M. aeruginosa* suspensions in MIR water; (3^{rd} Cl_2 Exp) cell-bound *M. aeruginosa* cellular material in ultrapure water; and (4^{th} Cl_2 Exp) natural cyanobacteria bloom samples from Missisquoi Bay. Each chlorination experiment was preceded by a chlorine decay experiment. For chlorine decay experiments, water samples were dosed with the desired chlorine concentration and triplicate samples were taken at specified contact times for free chlorine residual mesurements using the titration method. All chlorination experiments were conducted in triplicate in brown amber bottles at room temperature ($20 \pm 2^{o}C$).

Cultured *M. aeruginosa* samples were taken for preservation in Lugol's iodine solution before the enumeration of cells using a compound microscope (Nikon, Labophot-2, Phase contrast) in a Sedgewick-Rafter counting. Required volume of stationary phase culture to be spiked in ultrapure and MIR water to obtain the desired cell suspension was adjusted based on the culture cell number. The number of cells in water samples after inoculation was confirmed by microscopic enumeration. Control (non-chlorinated) water samples showed no sign of cell integrity loss due to direct addition of cells to ultrapure and MIR water. Detection of cells auto-fluorescence using the fluorescence microscopy method was used to assess the cells integrity. All chlorination experiments regardless of water quality parameters were conducted with a chlorine residual after 60 minutes of contact.

1^{st} Cl_2 Exp: These experiments were conducted in ultrapure water buffered at pH 8 with a 0.01 M phosphate buffer (KH_2PO_4/NaH_2PO_4) (American Public Health Association (APHA), et al., 2005). Chlorine was added from the chlorine stock solution to obtain Cl_2 doses of 2, 3 and 4.5 mg/L for *M. aeruginosa* suspensions of 60,000 (±2%), 250,000 (±4%) and 500,000 (±4%) cells/mL, respectively. Samples were quenched at various contact times (0, 1, 2, 5, 10, 15, 30 and 60 min) with sodium thiosulphate at a stoichiometric ratio specified in Standard Methods (American Public Health Association (APHA), et al., 2005). A portion of quenched samples (15 mL × 2) were taken through filteration for DOC measurement. To determine the cell lysis due to chlorination, total cell counts were conducted in triplicated on 2 mL samples after preservation in

Lugol's iodine (Wetzel & Likens, 2000). Remaining quenched water samples were divided into two subsamples of 250 mL: the first subsample was immediately filtered through 0.45 µm filter and then analyzed for extracellular MCs; the second subsample was taken to three freeze–thaw cycles before the filtration to analyze for total MCs (both the cell-bound and extracellular compounds). For all MCs analysis, confidence limits were calculated using the standard deviations of triplicate measurements. For all experiments, the extracellular concentrations of MCs were always within or below the confidence limit of total concentration. The differences between total and extracellular levels of MCs were estimated as cell-bound.

2nd Cl_2 Exp: These experiments were conducted in buffered MIR water with pH 7 and 8.5. Chlorine was added from the chlorine stock solution to achieve the Cl_2 doses of 2, 5 and 10 mg/L for *M. aeruginosa* suspension of 50,000 (±2%) cells/mL. Similar to "1st Cl_2 Exp" quenched samples were taken for DOC, extracellular MCs and total MCs measurements and cell lysis determination. . Cell densities (50,000 cells/mL corresponding to 0.12 mg/L DOC) were constant (±2%) and background MIR water DOC varied by less than 10%.

3rd Cl_2 Exp: These experiments were conducted on *M. aeruginosa* suspension of 500,000 (±2%) cells/mL in ultrapure water buffered at pH 8. Cultured cells were filtered and re-suspended in ultrapure water to eliminate the contribution of EOM. No sign of cell integrity loss was observed due to separation of cells from the culture media (data not shown). The *M. aeruginosa* suspension was subjected to three freeze–thaw cycles to release intracellular compounds before chlorination (McQuaid, et al., 2011). Chlorine was added from the chlorine stock solution to obtain the Cl_2 dose of 5.5 mg/L. Residual chlorine concentrations were determined after 30 and 1440 minutes of contact times and then samples were quenched with sodium thiosulphate. Quenched samples were taken for DOC and DBP measurements.

4th Cl_2 Exp: These experiments were conducted in Missisquoi Bay bloom water samples with 5.7×10^5 (±4%) cells/mL at ambient pH 7.8. Chlorine was added from the chlorine stock solution to obtain the Cl_2 doses of 2 and 5 mg/L. Similar to "1st Cl_2 Exp" quenched samples were taken for DOC, extracellular toxins and total toxins measurements and cell lysis determination.

2.4 Taxonomic counts with species identification

Taxonomic counts with species identification were performed using inverse microscopy (Lund, Kipling, & Le Cren, 1958; Wetzel & Likens, 2000) by the Centre d'Expertise en Analyse Environmentale du Québec (CEAEQ) at MDDEP. Temperature, pH, turbidity, as well as the initial and residual chlorine dosage values were collected from the records of the DWTP for the time period of concern.

2.5 Analysis of MCs and DBPs

A commercial Abraxis Microcystins ADDA ELISA Plate (an enzyme-linked immunosorbent assay – Abraxis LLC, Pennsylvania, USA) was used for MCs measurements. The analyses of MCs concentrations were measured as µg/L of MC-LR equivalent (MC-LR eq.). The detection limit for this assay is 0.1 µg/L as MC-LR.

DBP analyses were conducted by the laboratory of NSERC Industrial Chair on Drinking Water at Ecole Polytechnique de Montreal (Canada) using modified United States Environmental Protection Agency (USEPA) method 501.2 as described by Koch, Crofts, Schimpff, & Davis, 1988 (Koch, Crofts, Schimpff, & Davis, 1988) for THM analysis and USEPA method 552.2 for HAA. These samples were analyzed for four THMs (bromoform, chloroform, bromodichloromethane, and dibromochloromethane) as total THM (TTHM) and six HAA (bromoacetic acid, bromochloroacetic acid, chloroacetic acid, dibromoacetic acid, dichloroacetic acid, and trichloroacetic acid) as HAA6. They were measured using a Varian CP-3800 Gas Chromatograph (GC-ZCDs) with an electron capture detector (GC/ECD).

2.6 Data analysis

Chlorine exposure, CT, which is simplified by multiplying the residual concentration of chlorine by the exposure time as intermediate chlorine concentrations are not known (Equation 1) (Carrière, et al., 2010). The CT value is calculated by determining the area under a graph of chlorine concentration versus time (Ho, et al., 2006).

$$CT = \int_0^t [Cl]_t dt \qquad \text{Equation 1}$$

Where CT equals the chlorine exposure; t equals contact time; $[Cl]_t$ equals the chlorine residual at specified contact time.

The first-order rate constans (k_{Cl_2}) for chlorine decay were determined using Equation 2.

$$\ln \frac{[Cl]_t}{[Cl]_0} = - k_{Cl_2} t \qquad \text{Equation 2}$$

Where $[Cl]_t$ equals the concentration of free chlorine residual at specified contact time t; $[Cl]_0$ equals the concentration of free chlorine at contact time = 0; k_{Cl_2} equals the first order constants for chlorine decay; t equals contact time.

Reactions between chlorine and the *M. aeruginosa* cells and MCs are of second order, can also be described as first order to CT (Daly, et al., 2007; Lin, et al., 2009; A. Zamyadi, MacLeod, et al., 2012). In this study, cell lysis is defined by the reduction of cells resulting from sufficient cell damage to render undetectable under the microscope in a preserved sample. Equation 3 was used to calculate the toxin degradation or cell lysis apparent rate constants for these chlorination experiments (Daly, et al., 2007).

$$Ln\left(\frac{[X_{CT}]}{[X_0]}\right) = -k \times CT \qquad \text{Equation 3}$$

where CT equals the chlorine exposure; X_{CT} equals the number of lysed cells or MC-LR eq. concentration after a given chlorine exposure; X_0 equals the number of lysed cells or MC-LR eq. concentration at CT=0; and *k* equals the rate at which *M. aeruginosa* cells (k_{lysis}) or MC-LR eq (k_{appt}) oxidation occurs.

CHAPTER 3 RESULTS AND DISCUSSION

Results presented in this chapter include results that were included in the publication submitted to Water Research "Chlorination of *Microcystis aeruginosa*: Toxin release and oxidation, cellular chlorine demand and disinfection by-products formation" by authors Arash Zamyadi, Yan Fan, Rob I. Daly and Michèle Prévost. This publication uses the results produced by this experimental work. The results of this study are also included in a modelling effect which is not in the fram work of this thesis. The importance of my contribution is recognized by the fact that I am positioned as one of the authors. The article which has been accepted for publication in Water Research is presented in Appendix 4.

Also included in this chapter are results used for the publication Zamyadi et al 2012 (A. Zamyadi, MacLeod, et al., 2012). My results are specifically presented in the figures 4, 5, 6 and 7 in which I am a co-author of this publication "Toxic cyanobacterial breakthrough and accumulation in a drinking water plant: a monitoring and treatment challenge" which is presented in Appendix 5.

3.1 Impact of cyanobacterial celluler compounds and water matrix on chlorine demand

Free chlorine decay and cell lysis experiments were performed: (1) using cultured *M. aeruginosa* spiked in ultrapure water (60,000±2%, 250,000±4% and 500,000±4% cells/mL with 2, 3 and 4.5 mg/L of Cl_2 at pH 8, respectively) and natural MIR water (50,000±5% cells/mL with 2, 5 and 10 mg/L of Cl_2 at both pH 7 and 8.5); (2) using the Missisquoi Bay (MB) bloom water samples (approximately 5.7×10^5 (±4%) cells/mL with 2 and 5 mg/L of Cl_2 at ambient pH 7.8, pH augmented to 8.1 and 8.3 after chlorination with 2 and 5 mg/L Cl_2 respectively, and remained constant for the rest of the experiment) (Figure 3-1). Standard deviation of these cell enumerations (triplicate enumeration/sample) fit within the confidence limit of cell count method (Hurst, 2007; Laslett, Clark, & Jones, 1997; Lund, et al., 1958). Figure 3-1 presents the results of chlorine demand by time in these experiments. Chlorine demand in ultrapure water is obviously related to the *M. aeruginosa* cell numbers and the chlorine dose (Figure 3-1a). For the experiments with the same concentration of the *M. aeruginosa* cells in MIR water, chlorine demand in the absence of cells is lower than in the presence of cells with similar trends for both pH 7 and 8.5 (Figures 1b & 1c). In the MB bloom water samples at ambient pH, chlorine demand is directly related to the chlorine dose (Figure 3-1d). With the low chlorine dose of 2 mg/L, the MIR water (with and without the *M. aeruginosa* cells) consumed all chlorine after 30 minutes for both pH values (Figures 1b & 1c); the same result was observed in MB bloom water samples after 60 minutes (Figure 3-1d).

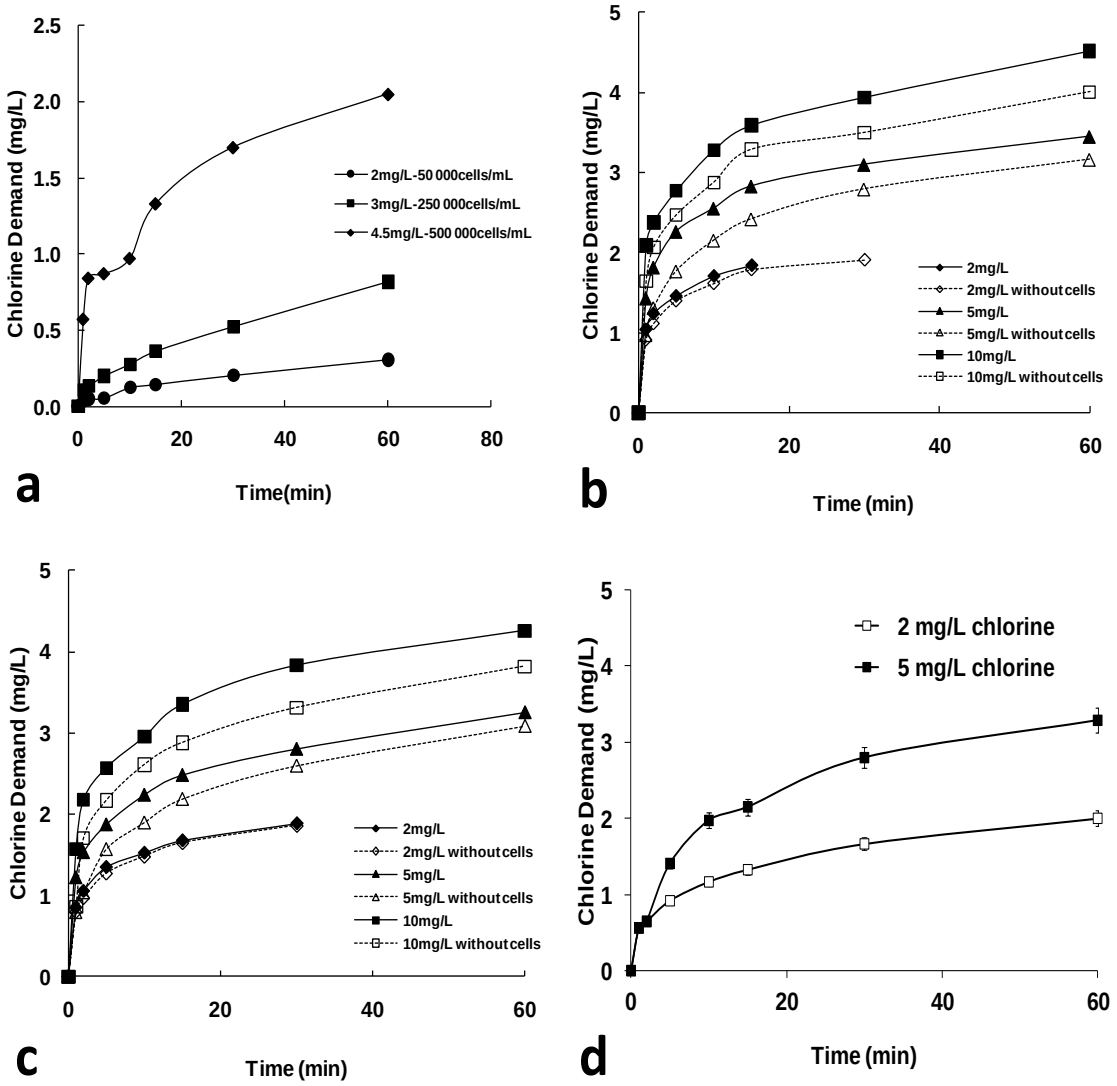

Figure 3-1: Chlorine demand of *M. aeruginosa* suspensions in (a) ultrapure water with 60,000, 250,000 and 500,000 cells/mL under 2, 3 and 4.5 mg/L Cl_2 respectively at pH 8, and 50,000 cell/mL in MIR water under 2, 5 and 10 mg/L Cl_2 (b) at pH 7, (c) pH 8.5, and (d) chlorine demand in Missisquoi Bay (MB) bloom water samples with 5.7×10^5 (±4%) cells/mL at ambient pH (pH ambient before chlorination was 7.8; pH augmented to 8.1 and 8.3 after chlorination with 2 and 5 mg/L Cl_2 respectively, and remained constant for the rest of the experiment)

Chlorine exposure (CT) value is the area under a plot of chlorine residual v.s. time, and describes the amount of free chlorine to which the solution has been exposed (Newcombe, et al., 2010). Chlorine decay data were used to calculate the CT values by fitting a two pathway integration model to a two phase decay curve: fast (< 1min) and slow route (Daly, et al., 2007; Sohn, Amy, Cho, Lee, & Yoon, 2004; A. Zamyadi, et al., 2010). The instant chlorine consumption observed within the first minute is described as the fast route; while the slow route represents the slower chlorine decay observed during the remaining chlorination. As shown in Figure 3-1, the free chlorine residual at 1minute showed almost a liner relationship to the initial free chlorine during the fast route.

Table 3.1 presents the resulting first order rate constants for chlorine decay. The slow apparent rate constants (k_{Cl2}) in ultrapure water without cells are very stable with all chlorine doses at pH 8 (Table 3.1). The addition of cyanobacteria cells to the ultrapure water raises the apparent rate constant values (Table 3.1). The k_{Cl2} values also augment with increasing cell numbers and chlorine doses. Besides, greater cell numbers were associated with higher DOC release (See section 3.4) coherent with the observed faster chlorine decay. In natural MIR water the apparent rate constants are very similar at the pH 7 and 8.5 but increase with the addition of cells and higher chlorine doses (Table 3.1). In MB bloom water samples, the first order rate constants for 2 and 5 mg/L of chlorine decay are 1.1×10^{-3} ($r^2 = 0.92$) and 1.0×10^{-3} ($r^2 = 0.97$), respectively.

Table 3.1: First-order apparent rate constants (k_{Cl2}) in s^{-1} for chlorine decay. All correlation coefficients (R^2) exceeded 0.85

Type of water	**Cell nb. (Cells/mL)**	**2 mg/L Cl_2**		**3 mg/L Cl_2**		**4.5 mg/L Cl_2**	
		pH 8		**pH 8**		**pH 8**	
Ultrapure	No cells	1.7×10^{-5}		1.6×10^{-5}		1.7×10^{-5}	
	60,000	6.4×10^{-5}		-		-	
	250,000	-		9.7×10^{-5}		-	
	500,000	-		-		1.5×10^{-4}	
	Cell nb. (Cells/mL)	**2 mg/L Cl_2**		**5 mg/L Cl_2**		**10 mg/L Cl_2**	
		pH 7	**pH 8.5**	**pH 7**	**pH 8.5**	**pH 7**	**pH 8.5**
MIR	No cells	8.4×10^{-5}	8.1×10^{-5}	1.8×10^{-4}	1.8×10^{-4}	1.1×10^{-3}	1.1×10^{-3}
	50,000	8.4×10^{-5}	8.2×10^{-5}	1.9×10^{-4}	1.9×10^{-4}	1.9×10^{-3}	1.9×10^{-3}
	Cell nb. (Cells/mL)	**2 mg/L Cl_2**		**5 mg/L Cl_2**			
		pH 8.1		**pH 8.3**			
MB	5.7×10^{5}	1.1×10^{-3}		1.0×10^{-3}			

3.2 Chlorination effect on cells lysis and estimation of chlorine demand per cell

In 1st Cl_2 Exp, the result of three *M. aeruginosa* cell suspensions of 60,000 (±2%), 250,000 (±4%) and 500,000 (±4%) cells/mL in ultrapure water buffered at pH 8 and chlorinated respectively at 2, 3 and 4.5 mg/L were evaluated (Figure 3-2). Figure 3-2a shows that chlorination in ultrapure water spiked with the lowest cell number (60,000±2% cells/mL) caused 60% of cell lysis after 30 minutes of contact (CT = 51 mg.min/L). Figure 3-2b reveals that chlorination in ultrapure water spiked with 250,000±4% cells/mL caused 57% of cell lysis after 60 minutes (CT = 107 mg.min/L). Figure 3-2c demonstrates that chlorination in ultrapure water spiked with highest cell number (500,000±4% cells/mL) caused 76% of cell lysis after 60 minutes (CT = 130 mg.min/L).

Additionally, the response of the *M. aeruginosa* cell suspensions of 50,000±5% cells/mL in MIR water at pH 7 and 8.5 to 2, 5 and 10 mg/L of Cl_2 was studied in 2nd Cl_2 Exp (Figure 3-3). The chlorination with the lowest chlorine dose of 2 mg/L caused 39% of cell lysis after 30 minutes (CT = 8.7 mg.min/L) at pH 7 and 40% of cell lysis after 30 minutes (CT = 13 mg.min/L) at pH 8.5. The chlorination with the higher chlorine dose of 5 mg/L caused 66% of cell lysis after 60 minutes (CT = 98 mg.min/L) at pH 7 and 67% of cell lysis after 60 minutes (CT = 111 mg.min/L) at pH 8.5. The chlorination with the highest chlorine dose of 10 mg/L caused 74% of cell lysis after 60 minutes (CT = 287 mg.min/L) at pH 7 and 75% of cell lysis after 60 minutes (CT = 296 mg.min/L) at pH 8.5. The results of cell lysis are very similar at pH 7 and pH 8.5 in MIR water.

Compared with Figure 3-2c in ultrapure water, Figure 3-3e and f showed that similar levels of cell lysis (~75%) were achieved with the same or higher CT values in MIR water. In a study of chlorination of 46,000 cell/mL of *Anabaena circinalis* suspension, the reduction in total cell numbers with a CT of ~50 mg.min/L was only around 15% (A. Zamyadi, et al., 2010). However, they also reported that over 98% of cells (even detectable under microscope in preserved samples) could be categorized as injured/dead cells (damaged membrane or metabolically inactive cells) at a CT < 7 mg.min/L. It is important to consider that cell lysis, here defined as corresponding to the loss of detectable stained cell structures, is a less conservative indicator of loss of viability as it occurs at the last step of cell decay.

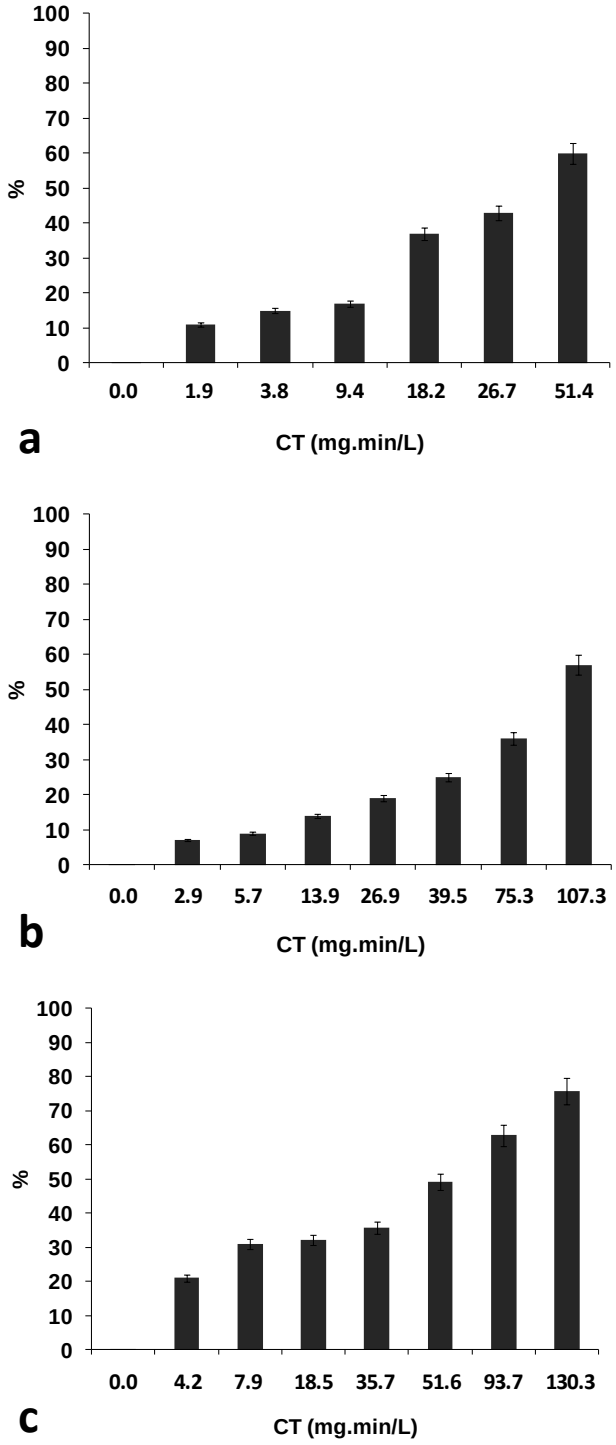

Figure 3-2: Reduction in number of cells (cell lysis) during chlorination of *M. aeruginosa* suspensions of (a) 60,000 cells/mL with 2 mg/L Cl_2, (b) 250,000 cells/mL with 3 mg/L Cl_2, and (c) 500,000 cells/mL with 4.5 mg/L Cl_2, in ultrapure water at pH 8

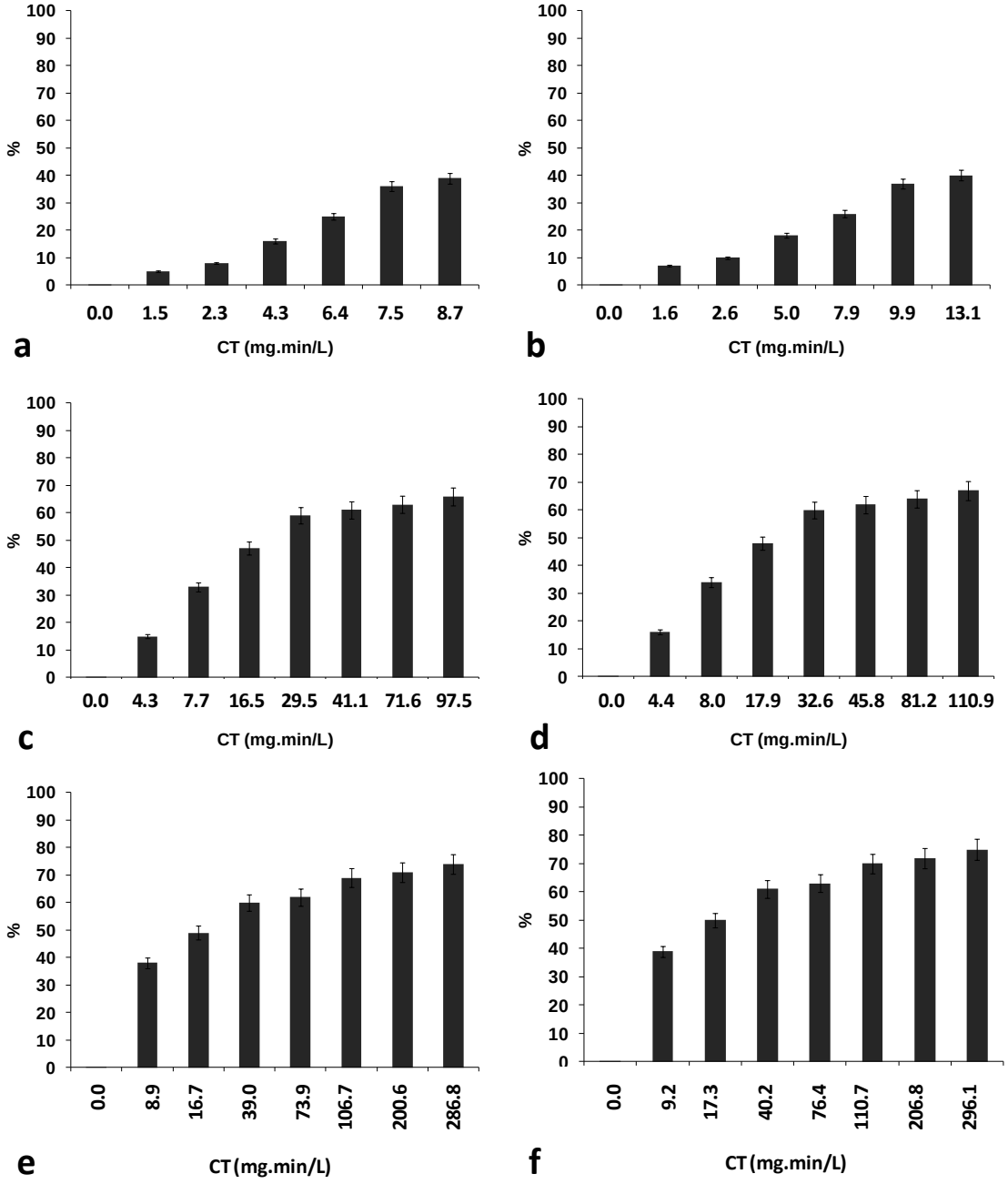

Figure 3-3: Reduction in number of cells (cell lysis) during chlorination of *M. aeruginosa* suspensions (50,000 cells/mL) in MIR water (a) at pH 7 with 2 mg/L Cl_2, (b) at pH 8.5 with 2 mg/L Cl_2, (c) at pH 7 with 5 mg/L Cl_2, (d) at pH 8.5 with 5 mg/L Cl_2, (e) at pH 7 with 10 mg/L Cl_2, and (f) at pH 8.5 with 10 mg/L Cl_2

The assumption of a first-order reaction with respect to chlorine reaction and the *M. aeruginosa* cells lysis is valid (Daly, et al., 2007; Lin, et al., 2009). Hence, the reaction between chlorine and the cells is of second-order and the constant rate at which cell lysis occurs (k_{lysis}) can be evaluated using Equation 3. The k_{lysis} calculated for chlorination of *M. aeruginosa* cells are presented in Table 3.2. These k_{lysis} values were similar in both experiments conducted in ultrapure and natural MIR water.

The k_{lysis} values in MIR water are close to chlorination lysis rates estimated for laboratory cultures of *M. aeruginosa* (A. Zamyadi, Ho, et al., 2012) (Table 3.2). In other studies, the rates of cell membrane damage, also referred to as the loss of cell integrity after chlorination ($k_{release}$) are estimated using cell integrity tests. $k_{release}$ represents the release of cell bound material even if the cells are still present in water and is not for directly compare with k_{lysis} in this study. $k_{release}$ from cultured cells in chlorination assays have been found to be higher compared to those measured for cells from natural blooms (Lin, et al., 2009; A. Zamyadi, Ho, et al., 2012). In other cases, $k_{release}$ values for cultured cells fall within the range of k_{lysis} values estimated in this work. Many factors, such as the physiological state of the cyanobacterial cells, the cell densities, the water matrices and the chlorination conditions, could cause different response in susceptibility during chlorination (Lin, et al., 2009; Pietsch, et al., 2002).

M. aeruginosa cellular chlorine demand per cell was calculated from the lysis of *M. aeruginosa* suspensions in ultrapure water in the presence of chlorine residual (Figure 3-1a) (Figure 3-2). The cumulative chlorine demand after 60 minutes of contact per number of lysed cells is almost a constant value of 5.6±0.2 $pgCl_2$/cell for the three experiments conducted in buffered ultrapure water. This novel information about the value of unit cellular chlorine demand should be very useful and practical for DWTP operators. It can help to estimate if the chlorination capacity is sufficient to satisfy the total chlorine demand associated with the presence of cyanobacteria.

Table 3.2: Apparent rate constants for cell lysis or reduction in total cell count (k_{lysis} - $M^{-1}s^{-1}$), for loss of cell integrity or cell rupture ($k_{release}$ - $M^{-1}s^{-1}$), for total toxins oxidation (k_{total} - $M^{-1}s^{-1}$) and for the oxidation of dissolved toxins released from damaged cells ($k_{dissolved}$ - $M^{-1}s^{-1}$) derived from this study compared to published results. Comments in parenthesis describe the origin of the CB cells (from laboratory cultures or environmental bloom samples) and the toxins (from standard extracts or cell-bound) (* MC-LR equivalent)

	This study										**Published studies**						
	60,000 cells/mL, Ultrapure water, 2 mg/L Cl_2, pH 8	250,000 cells/mL, Ultrapure water, 3 mg/L Cl_2, pH 8	500,000 cells/mL, Ultrapure water, 4.5 mg/L Cl_2, pH 8	50,000 cells/mL, MIR water, 2 mg/L Cl_2, pH 7	50,000 cells/mL, MIR water, 2 mg/L Cl_2, pH 8.5	50,000 cells/mL, MIR water, 5 mg/L Cl_2, pH 7	50,000 cells/mL, MIR water, 5 mg/L Cl_2, pH 8.5	50,000 cells/mL, MIR water, 10 mg/L Cl_2, pH 7	50,000 cells/mL, MIR water, 10 mg/L Cl_2, pH 8.5	5.7×10^5 cells/mL, MB bloom sample, 2 & 5 mg/L Cl_2, pH 8.3	(Acero, et al., 2005). pH 7.9-8	(Ho, et al., 2006). pH 7.9	(E. Rodriguez, Onstad, et al., 2007). pH 8	(Daly, et al., 2007).pH 6.8-7.6	(Lin, et al., 2009),pH 8.3-8.6	(Ding, Shi, Timmons, & Adams, 2010).2010.pH 7.6	(A. Zamyadi, Ho, et al., 2012).pH 8
M. aeruginosa **k_{lysis} ($M^{-1}s^{-1}$)**	27.9	17.9	11.6	234.1	170.2	73.1	33.3	30.6	11.5	3.8-16 bloom	-	-	-	-	-	-	37 (culture)
M. aeruginosa **$k_{release}$ ($M^{-1}s^{-1}$)**	-	-	-	-	-	-	-	-	-	-	-	-	-	670±77	790-1100 (culture), 70-590 (bloom)	43.6 (culture)	756-1030 (culture)
k_{total} MC-LR	33.5 *	49.6 *	88.7 *	-	-	-	-	-	-	2.1-17.9* bloom	-	-	-	10-96 (cell-bound)	-	-	21-23.3 (cell-bound)
$k_{dissolved}$ MC-LR	-	-	20.2 *	-	-	-	-	-	-	-	33.1-38.0	110-170	33	242	-	55.9	-

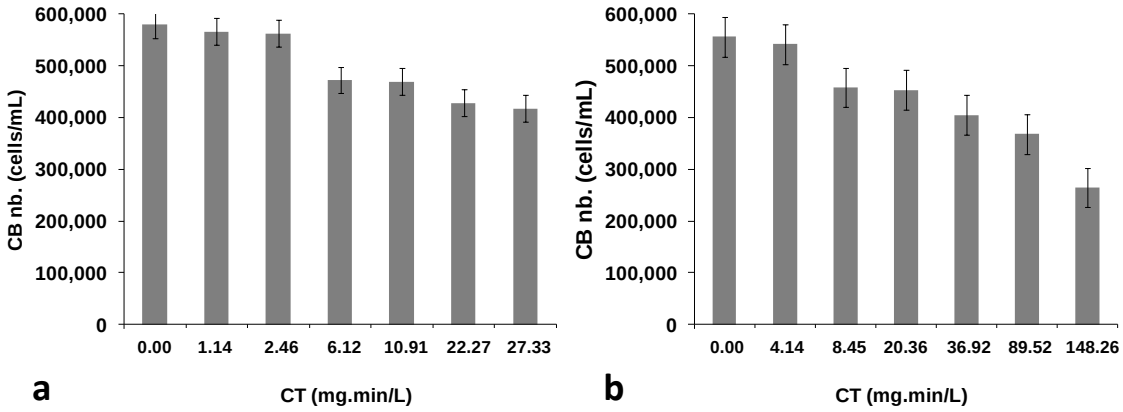

Figure 3-4: Cell numbers after chlorination of Missisquoi Bay bloom water samples using (a) 2 mg/L Cl_2, and (b) 5 mg/L Cl_2 (Adapted from (A. Zamyadi, MacLeod, et al., 2012))

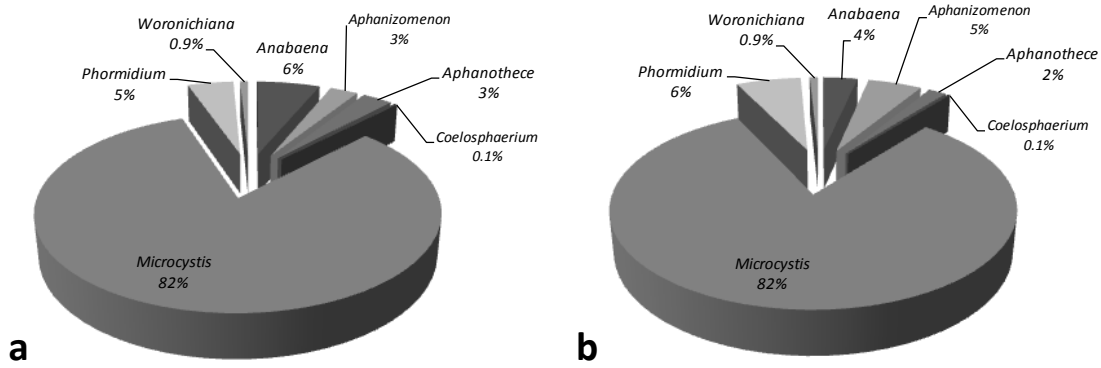

Figure 3-5: Proportion of seven cyanobacteria (CB) species out of total cyanobacteria cell in Missisquoi Bay bloom sample (a) before chlorination (5.7×10^5 (±4%) cells/mL), and (b) after chlorination using a CT of 148 mg.min/L (2.7×10^5 (±4%) cells/mL – 53% reduction of total cell numbers) (from (A. Zamyadi, MacLeod, et al., 2012))

Furthermore, the result of the MB bloom water samples (approximately 5.7×10^5 ±4% cells/mL) to chlorine doses of 2 and 5 mg/L at ambient pH was evaluated. After 60 minutes of chlorination for both chlorine doses, the reductions of total number of cells, corresponding to full cell lysis, were 28% to 53% (Figure 3-4). However, these MB bloom water samples incorporated seven different cyanobacteria species but dominated by *Microcystis* sp (Figure 3-5). Besides, after the chlorination with a CT of 148 mg.min/L and a 53% reduction in total cell number, the proportion of cyanobacteria species which regards to total number of cells present was almost the same as before chlorination with slight declines in *Anabaena* sp. and *Aphanothece* sp (Figure 3-5). It suggests assuming a second-order reaction with regards to chlorine and the reduction of total cell numbers in the MB bloom samples, the k_{lysis} calculated using Equation 3 for chlorination of MB bloom water samples are presented in Table 3.2 as well. The k_{lysis} values in MB bloom water samples are close to the k_{lysis} values in MIR water with laboratory culture of *M. aeruginosa*; but they are in disagreement with the results of previous studies (Table 3.2). The susceptibility of the cells of the CB species to chlorination is also affected by the physiological state of the cells, cell densities, water matrix, and the chlorination conditions (Hart, et al., 1998; Lin, et al., 2009; Pietsch, et al., 2002).

3.3 Impact of chlorination on release and oxidation on cell-bound cyanotoxins

In previous studies (Daly, et al., 2007; Lin, et al., 2009; A. Zamyadi, et al., 2010), fast release of cell-bound compounds, especially cell-bound toxins, have been observed when pre-chlorinating (with low chlorine doses as 1-5 mg/L Cl_2) natural water loaded with *Anabaena* sp. and *Microcystis* sp. In this study, Figure 3-6 presents the release and oxidation of cell-bound MCs (MC-LR eq.) in buffered ultrapure water at pH 8 for the *M. aeruginosa* suspensions of 60,000 (±2%), 250,000 (±4%) and 500,000 (±4%) cells/mL. There is a strong relationship between chlorine exposure and the total toxins degradation, and also with a quick release of cell-bound toxins in Figure 3-6b and Figure 3-6c. With a CT value of 100 mg.min/L, Figure 3-6 presents a complete toxin release and reduction below the WHO guideline value (1 µg/L MC-LR for drinking water) oxidation of the toxins. The observed toxins release and oxidation (Figure 3-6) agree well with cell lysis (Figure 3-2).

Furthermore, Figure 3-7 shows the toxins release and oxidation after chlorination of MB bloom water samples (approximately 5.7×10^5 ±4% cells/) using 2 and 5 mg/L Cl_2. The cell-bound toxins are still observed and are much higher than the WHO guideline value even after the highest CT value of 148 mg.min/L. DOC variations before and after chlorination were <0.2 mg/L C.

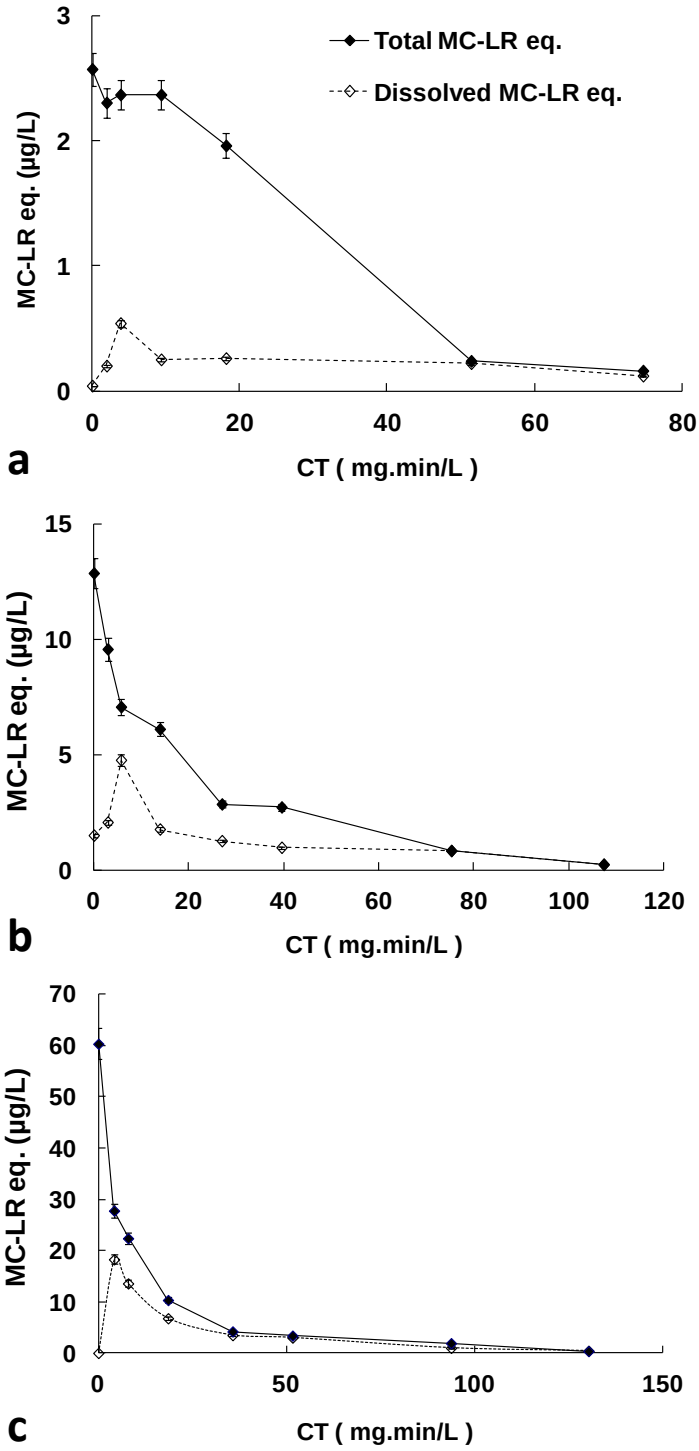

Figure 3-6: Toxins release and oxidation from *M. aeruginosa* suspensions in ultrapure water at pH 8 with cell number and chlorine doses of (a) 60,000 cells/mL and 2 mg/L Cl_2, (b) 250,000 cell/mL and 3 mg/L Cl_2, and (c) 500,000 cells/mL and 4.5 mg/L Cl_2, respectively

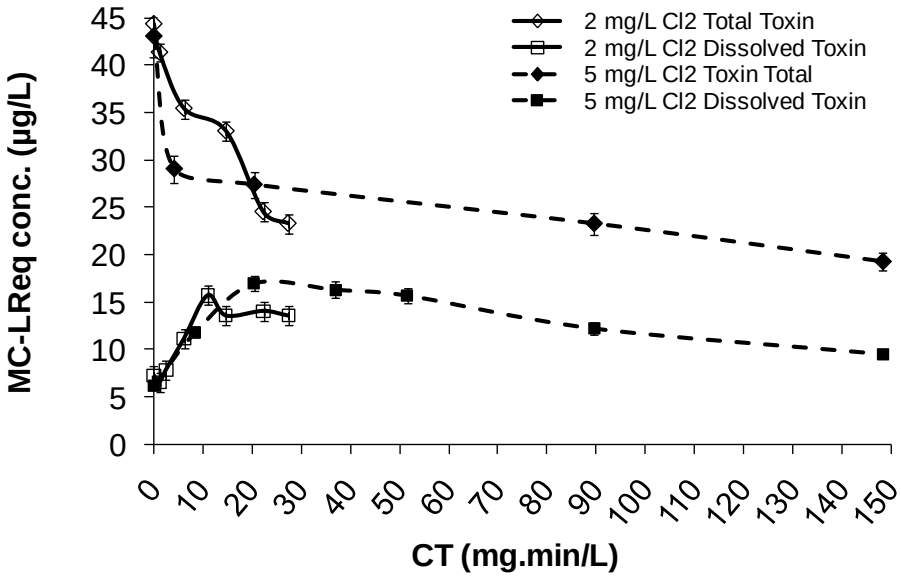

Figure 3-7: Toxins release and oxidation after chlorination of Missisquoi Bay bloom water samples (approximately 5.7×10^5 ±4% cells/mL) using 2 and 5 mg/L Cl_2 (from (A. Zamyadi, MacLeod, et al., 2012))

Kinetic models can express the chlorination of released cell-bound and dissolved MCs (Daly, et al., 2007; Lin, et al., 2009). Second-order rate constants (k_{total}) represent the combined rate of the release of cell-bound toxins due to cell lysis and the oxidation of the released toxins. For the oxidation of total MC-LR eq. in both ultrapure water and MB bloom water samples, k_{total} can be estimated using Equation 3. When using dense suspensions of 500,000 cells/mL of *M. aeruginosa* in ultrapure water (Figure 3-6c), it is possible to estimate the rate constant for the dissolved toxins ($k_{dissolved}$) after complete release as indicated by the elevated cell lysis. This estimate of $k_{dissolved}$ model may be considered as more representative of conditions of oxidation of dissolved toxins in the presence of cellular debris, as would be the case during a bloom.

The calculated apparent rate constant $k_{dissolved}$ from this study and previously published rate values are listed in Table 3.2. Our estimate of $k_{dissolved}$ for MC-LR eq. is equivalent or lower than those published for spiked toxins in ultrapure and natural waters (Acero, et al., 2005; Ding, et al., 2010; E. M. Rodriguez, et al., 2008). The reported values of $k_{dissolved}$ vary by 7.3 times with the highest values observed in spiked natural water or in the presence of elevated densities of cells. Differences can be explained partly by slight variations in experimental pH as the oxidation of MC-LR which is more efficient at pH values below 8 (Ho, et al., 2006). This phenomenon has been contributed to the degree of MC-LR protonation at lower pH values making it more

susceptible to chlorination (Ho, et al., 2006). The impact of NOM can also be significant because of organic matter-MC interactions or the action of highly reactive quaternary chloramines on OM moieties (Ho, et al., 2006).

Our observations support previous observations of rapid oxidation of cell-bound toxins upon released from damaged or lysed cells. In most cases, chlorination causes a fast release of cell-bound toxins as shown by the $k_{release}$ values. Overall, the oxidation of toxins, whether ($k_{total)}$ or dissolved ($k_{dissolved}$) occurs somewhat more quickly than cell lysis (k_{lysis}), suggesting that released cell-bound toxins react faster with chlorine the damaged cells and debris. In some cases, the differences are notable, while in others insignificant. However, larger differences have been reported for MC-LW, MC-LF, MC-RR, MC-YR and MC-LA chlorination rates (3320, 204, 136, 94 and 89.5 $M^{-1}s^{-1}$ respectively) as compared to cell inactivation rate (43.6 $M^{-1}s^{-1}$) (Ding, et al., 2010). Greater differences are expected when comparing toxin oxidation rates with cell lysis rates, as cell lysis is a more conservative indicator than gradual cell membrane damage and loss of cell viability as previously observed by A. Zamyadi et al., 2010. (A. Zamyadi, et al., 2010).

3.4 Chlorination effect on cellular organic carbon release and DBP formation

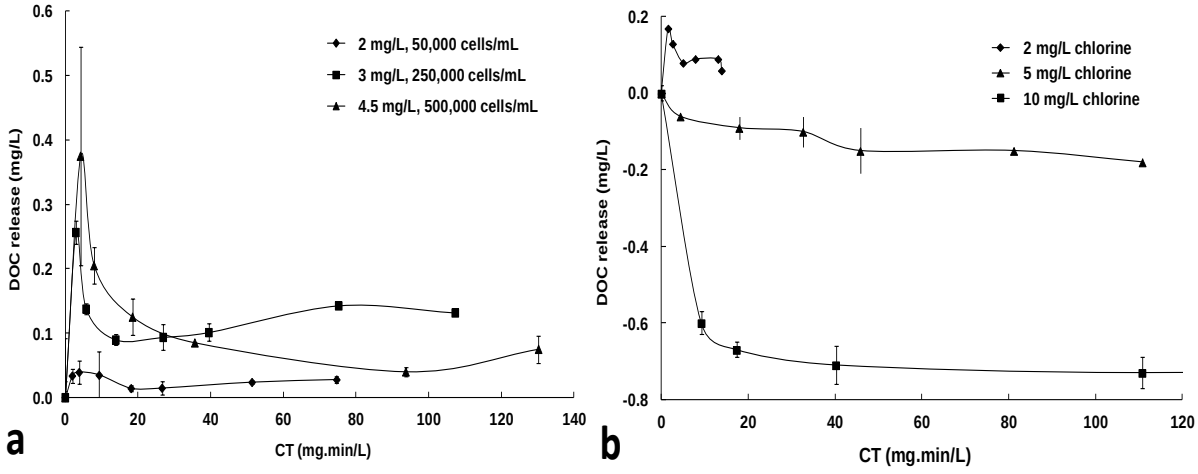

Figure 3-8: DOC release from chlorination of *M. aeruginosa* suspensions in (a) ultrapure water at pH 8 and (b) MIR water at pH 8.5 spiked with 50,000 cells/mL (results in MIR water at pH 7 were similar to those of pH 8.5)

During the chlorination, cyanobacterial cells could be demaged by chlorine and subsequently release intercellular DOC (Miao & Tao, 2009). Figure 3-8 demonstrates the effect of chlorination on DOC with increasing concentrations of *M. aeruginosa* suspensions in ultrapure and MIR water at pH 7 to 8.5. As shown in Figure 3-8a, the final DOC of cellular suspensions in ultrapure water augmented after 60 minutes of chlorination compared to their initial DOC. The maximum DOC release occurred rapidly within the first quenching time (1 minute with a maximum CT of 4.2 mg.min/L in chlorination of 500,000 cells/mL with 4.5 mg/L of Cl_2). This release corresponds to a fraction (<10%) of the total carbon cell content estimated at 1.1 mg/L for 50,000 cells/mL equivalent (A. Zamyadi, Ho, et al., 2012). The maximum released concentrations reflect the increasing concentrations of cells added. The release is immediately followed by a rapid decrease in DOC concentrations (Figure 3-8a) which is most possibly due to the flocculent action of the hydrophilic polysaccharides released from the damaged cells (R. K. Henderson, Baker, Parsons, & Jefferson, 2008).

In Figure 3-8b, during chlorination of *M. aeruginosa* suspension (50,000 cells/mL) in MIR water, DOC variations with 2 mg/L of Cl_2 followed a similar trend to that observed in ultrapure

experiments. On the other hand, this trend is greatly enhanced when applying a higher dosage of chlorine (as 5 and 10 mg/L) to the cellular suspensions of 50,000 cells/mL in natural MIR water. There was a net decrease of DOC up to 0.73 mg/L C from the background DOC values of 6.3 mg/L of the MIR water (Figure 3-8b). Similar trends were observed at a buffered pH of 7. This further proves the hypothesis that increasing dosages is in favor of the formation of polysaccharides based compounds that shift AOM and humic acids from the natural water to particulate NOM. Unfortunately, total organic carbon measurements have not been conducted which would have reconsolidated the data by showing the organic matter change to particulate fraction.

The DBP formation is a negative consequence for chlorination of cyanobacteria, especially THMs from the reaction of chlorine with NOM present in the nature water (Acero, Rodriguez, Majado, Sordo, & Meriluoto, 2008). Furthermore, some studies have shown that cell-bound DOC can be a significant source of DBP precursors (Huang, et al., 2009; Nguyen, et al., 2005; J. D. Plummer & Edzwald, 2001; Wardlaw, Perry, & Graham, 1991).

In 3rd Cl^2 Exp, the reactivity of cyanobacterial organic matter, whether dissolved or particulate, was further eveluated. To specifically investigate the contribution of *M. aeruginosa* cells to DBP formation during chlorination, the cells were filtered and re-suspended in ultrapure water, thus eliminating the contribution of EOM. Depending on the cell growth phase and culture conditions, the concentration of EOM release and accumulation can vary broadly, which can reach up to of 40 mg/L C in extremely dense suspensions (Hoyer, Lusse, & Bernhardt, 1985; Nguyen, et al., 2005). In order to release IOM, a dense suspension of 500,000 cells/mL was subjected to three freeze–thaw cycles prior to chlorination with 5.5 mg/L Cl_2 at pH 8. Table 3.3 presents the TTHM and HAA6 formation in the compromised *M. aeruginosa* suspension after 24 hours. The measured concentrations of TTHM and HAA6 after the maximum CT of 3051 mg.min/L are still under the current reference standard of 80 µg/L for TTHM and 60 µg/L for HAA5 (United States Environmental Protection Agency (USEPA), 2006).

The results of this study are contrasting with those from Huang et al., 2009 (Huang, et al., 2009) which reported high yields of THM and HAA precursors from high density suspensions of cultured *Microcystis* sp. with a very high DOC during a chlorination experiments (pH 7 with over 0.5 mg/L Cl_2 residual from 1 to 7 days of contact). The estimated biovolume of *M. aeruginosa* is

53 μm^3/cell and its maximum estimated dry weight is 44 pg/cell (Reynolds, 1984). Optimum content of carbon (C) in a *M. aeruginosa* cell is approximately 50% of the cell dry weight (Reynolds, 1984). Thus, the carbon content of our suspension of 500,000 cells/mL of *M. aeruginosa* (suspension with highest cell number) is around 11 mg/L as C. The highest published chloroform yield for chlorination of *Microcystis* sp. is 61 µg $CHCl_3$/mg C (Huang, et al., 2009). Hence, 671 µg/L is the maximum potential THM formation for our *M. aeruginosa* suspension of 500,000 cells/mL. In this study, the measured DOC of the suspension of 500,000 cells/mL of *M. aeruginosa* is only 1.3 mg/L as C. Actual THM yield from the chlorination of IOM and EOM of this cell suspension within a contact time of 24 hours (Table 3.3) is below the maximum THM potential formation values. The published maximum potential yield of HAA precursor for intense *Microcystis* sp. culture is smaller than the published values for chloroform (Huang, et al., 2009). However, the actual HAA6 measured in chlorination of IOM and EOM of 500,000 cells/mL of *M. aeruginosa* after 24 hours was below detection limit (Table 3.3).

Table 3.3: DBP formation in chlorination of lysed *M. aeruginosa* suspension of 500,000 cells/mL in ultrapure water at pH 8

Time (min)	**Cl_2 residual (mg/L)**	**Cl_2 demand (mg/L)**	**CT (mg.min/L)**	**TTHM (µg/L)**	**HAA6 (µg/L)**
0	5.5	-	0.00	BDL*	BDL
30	2.9	2.6	125.4	BDL	BDL
1440	1.3	4.2	3051.2	12.87	BDL

* Below Detection Limits (BDL)

The *M. aeruginosa* cells used for this study were harvested only at stationary phase of growth. The growth phase can influence the types of protein and carbohydrate associated with cyanobacterial cells (R. Henderson, Parsons, & Jefferson, 2008; R. K. Henderson, et al., 2008); thus varying the reactivity of these compounds with chlorine and their DBP formation potentials. However, the results of 3rd Cl^2 Exp showed that the chlorination of 500,000 cells/mL and less of M. aeruginosa cells did not contribute importantly to the DBP formation.

CONCLUSION

Results of this research show that chlorine demand is directly related to cyanobacteria (*M. aeruginosa*) cell numbers and the chlorine doses when chlorination in ultrapure water, in MIR water and even in the natural MB bloom water samples. The first-order rate constant for chlorine decay (k_{Cl_2}) also increased with increasing cyanobacteria cell numbers and chlorine doses. Furthermore, greater cell numbers are associated with higher release of DOC coherent with faster chlorine decay. The results of chlorine demand with *M. aeruginosa* in MIR water under different pH 7 and 8.5 are similar and keep the same trends.

This research also demonstrates that chlorine doses of 2 to 10 mg/L cause the fast release and subsequent oxidation of dissolved and cell-bound MCs from *M. aeruginosa*. Additionally, a maximum of 76% reduction in total cell numbers (cell lysis) was observed with a CT of 296 mg/min/L. A rather constant value of of 5.6±0.2 pgCl_2/cell is estimated as the *M. aeruginosa* cellular chlorine demand when cell lysis occurs due to chlorination. The cellular chlorine demand in the presence of cellular debris is the key information provided in this paper to the operators of DWTPs to adjust their chlorination practice. This information will be directly relevant to water authorities and DWTP operators to validate the efficiency of chlorination barrier.

There is DOC release during the chlorination and the maximum DOC release occurs very quickly within the first minutes. For the study of DBP formation, these results show that chlorination of 500,000 cells/mL and less of *M. aeruginosa* cells do not contribute significantly to the DBP precursor's pool. However, the background NOM and its DBP formation potential will determine whether the chlorination of *M. aeruginosa* cells can be considered without the formation of excessive chlorination by-products.

The results about the MB bloom samples show that 28% to 53% reductions of total number of cells, corresponding to full cell lysis, were observed after 60 minutes of chlorination. Comparing the results of cell lysis of laboratory *M. aeruginosa* culture, natural blooms are more resistant than cell suspension of laboratory cultures. Low chlorine doses of 2 and 5 mg/L Cl_2 were not enough to oxidize the toxins of the MB bloom sample and to achieve the WHO guideline value even after 60 minutes. DOC variation before and after chlorination were below 0.2 mg/L C. Furthermore, these results demonstrate that breakthrough of cyanotoxins at concentration

exceeding the health-based exposure alert levels can occur, even for toxins considered to be readily oxidized by chlorine.

Even though pre-chlorination is a promising barrier against cyanobacteria and their toxins accumulation and breakthrough in drinking water treatment process, it is essential to be cautions with the exposition of experimental results and the application in the practical application.

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APPENDICES

APPENDIX 1: Cyanotoxin name, producer organisms and health effects

(Adapted from (Chorus & Bartram, 1999; Hitzfeld, et al., 2000; Svrcek & Smith, 2004; Arasah Zamyadi, 2011)

Toxin group		Toxin variant	Toxic	Producer cyanobacteria genera	Health effect
Cyclic peptides		Microcystins(MCs): MC-LR, MC-RR MC-YR, MC-LA MC-LF, MC-LW	Hepatotoxic	*Anabaena, Anabaenopsis,Aphanicapsa, Hapalosiphon, Microcystis,Nastoc, Oscillatoria*	Abdominal pain, diarrhea, vomiting, blistered mouth, dry cough, headache, painful breathing, pneumonia. Liver damage. Tumor promoter: The relationship between the animal tumor growth promotion properties of these toxins and human carcinogenicity needs to be determined.
		Nodularins(NODs)	Hepatotoxic	*Nodularia* (mainly brackish water)	Liver damage and tumor promoter.
Alkaloids	*Neurotoxic alkaloids*	Anatoxin-a	Neurotoxic	*Anabaena, Aphanizomenon, Oscillatoria*	Post synaptic depolarizing neuromuscular blocking agent. Staggering, muscle fasciculation, gasping.
		Anatoxin-a(S)	Neurotoxic	*Anabaena, Oscillatoria*	Cholinesterase inhibitor. Hyper salivation.
		Saxitoxin(STX): C-toxins Gonyautoxins(GTXs) Saxitoxins	Neurotoxic	*Anabaena, Aphanizomenon, Cylindrospermopsis, Lyngbya*	Sodium-channel blockers. Acute poisoning results in death by paralysis and respiratory failure.
	Cytotoxic alkaloids	Cylindrospermopsin	Cytotoxic,Hepatotoxic Neurotoxic,Geotoxic	*Anabaena, Aphanizomenon, Cylindrospermopsis,Umezakia*	Liver, kidney and other organ damage. Protein synthesis inhibition.
	Dermatotoxic allaloids	Aplysiatoxin	Dermatotoxic	Maine cyanobacteria *Lyngbya, Schizothrix, Oscillatoria*	
		Debromoaplysiatoxin	Dermatotoxic	*Lyngbya, Schizothrix, Oscillatoria*	
		Lyngbyatoxin-a	Dermatotoxic	*Lyngbya*	Oral and gastro-intestinal inflammation.
Lipopolysaccharides (LPS)			Endotoxic	Most cyanobacteria	Potentially irritates any exposed tissue: Skin, eye irritation, skin rashes.

APPENDIX 2: Measurement methods for cyanotoxins

There are some general considerations for optimum measurement method of cyanotoxins. In water bodies, bloom-forming cyanobacteria will present their toxins in both the water as dissolved extracellular toxins and the cyanobacterial cells as intracellular toxins at the same time (Chorus & Bartram, 1999; Pietsch, et al., 2002). These current analytical techniques only can measure the toxins in the free or dissolved state (B. Nicholson & Shaw, 2011). To measure the total levels of toxins in the samples containing healthy blooms, cyanobacterial cells must be lysed to release intracellular toxins, usually by freeze-drying followed by sonication in solution, or repeated freeze-thawing and/or sonication of whole cell material (Svrcek & Smith, 2004). Therefore the extraction of intracellular toxins should be considered firstly, as the basis of any measurement procedure. The concentrations, stability of cyanotoxins and the goal of sampling program must also be considered for choosing the method (B. C. Nicholson & Burch, 2001).

Table 1: Summary of common analytical measurement methods for cyanotoxins (Adapted from (B. Nicholson & Shaw, 2011; B. C. Nicholson & Burch, 2001; Svrcek & Smith, 2004))

Toxin	Analytical Method	Detection Limit	Comments
Microcystins	ELISA	0.05 µg/L, no preconcentration	Screening assay for total microcystins, as microcystin-LR equivalents if microcystin-LR is used for calibration; Low level of expertise required; Operational costs high as commercial kits expensive and have limited shelf-life; May underestimate toxin concentration.
	HPLC-UV/PDA/MS	0.02 µg/L for individual toxins using a 5 L sample, depends on concentration factor	HPLC is the most common instrumental detection method for microcystins; Moderate level of expertise required, high level to correctly identify microcystins; Relatively expensive to set up and operate.
	MMPB method	0.43 ng microcystin, depends on concentration factor	Screening assay for a sum total of microcystins, as microcystin-LR equivalents if microcystin-LR is used for calibration; Microcystins oxidized, Adda side chain converted to MMPB; Moderate level of expertise; Relatively inexpensive.
	PPIA colorimetric	0.3 µg/L, no preconcentration	Screening assay for total microcystins if used for calibration; Low to moderate level of expertise required; Relatively inexpensive; No identification of individual toxins.
	Mouse bioassay	1 to 200 µg	Screening assay; Qualitative results.
Nodularin	ELISA	0.05 µg/L	Semi-quantitative screening assay.
	HPLC	0.5 µg/L, < 1 µg/L	Provides a spectrum of the separated analytes.
	PPIA	0.1 µg/L	Screening assay; Relatively simple; Highly sensitive.
	Mouse bioassay	1 to 200 µg	Screening assay; Qualitative results.
Cylindrospermopsin	ELISA	0.05 µg/L	Semi-quantitative screening assay capable of detecting low toxin concentration.
	LC/MS/MS	1 µg/L	High degree of compound specificity; No need for sample preparation.
	Mouse bioassay	1 to 200 µg	Screening assay; Qualitative results.
Saxitoxins	ELISA	0.02 µg/L, depends on antibodies used in assay	Semi-quantitative screening assay; Has advantage of detection of low levels STX; Poor cross reactivity to some analogues, Moderate expertise required.
	HPLC	Depends on concentration factor	Detection limits of STX have been determined using HPLC with post-column derivation and fluorescence detection (without sample concentration); Moderate expertise required.
	Mouse bioassay	1 to 200 µg	Screening assay; Qualitative results.

Note: ELISA, enzyme-linked immunosorbent assays; HPLC, high performance liquid chromatography; UV, ultraviolet; PDA, photo-diode array; MS, mass spectrograph or mass spectral; MMPB method, 3-menthoxy-2-methyl-4-phenylbutyric acid method; PPIA, protein phosphatase inhibition assay.

APPENDIX 3: Conventional treatments of cyanobacteria

Under suitable conditions, coagulation and flocculation can promote the aggregation of small, dispersed cyanobacterial particles into larger ones for further removal by separation and filtration (Svrcek & Smith, 2004). Efficient removal is dependent on optimization of applicable chemicals (i.e. various aluminum, ferric iron salts and synthetic polymers), chemical doses, and pH (Chorus & Bartram, 1999; De Julio, Fioravante, De Julio, Oroski, & Graham, 2010). In many literatures, coagulation and flocculation were effective at 3-log removal of cyanobacterial cells without significant damage to membrane integrity (Drikas, Newcombe, & Nicholson, 2001). A study reported that chemical treatment and mechanical agitation did not damage the cultured *M. aeruginosa* cells and, more importantly, did not result in additional release of cell metabolites above background concentrations (Chow, et al., 1999). Some studies found that coagulation and flocculation removed intracellular toxins via the removal of the cells themselves; however they did not effectively remove dissolved toxins (Chorus & Bartram, 1999). Another study used three coagulants: ferric sulphate, alum and polyaluminium chloride to remove dissolved microcystins; in all cases there was virtually no toxin removal (B. C. Nicholson, et al., 1994).

Although coagulation/flocculation is normally followed by a sedimentation, in cyanobacteria-rich waters, flotation such as dissolved air flotation (DAF) is more effective than sedimentation (Knappe et al., 2004). DAF gives better results for the removal of intact cyanobacterial cells, particularly for those low-density species with gas vacuoles that may render them more difficult to settle: 40 to 80% removal of *Microcystis*, 90 to 100% removal of *Anabaena* but only 30% for *Planktothrix* (syn *Oscillatoria*) (Mouchet & Bonnelye, 1998; Newcombe, et al., 2010). However, it's questionable that DAF process is more active than sedimentation for removing extracellular toxins. Toxins release and sludge treatment need to be evaluated further.

Filtration which requires regular backwashing to maintain performance, is effective for the removal of intracellular toxins (Chorus & Bartram, 1999). Direct rapid filtration without prior chemical treatment (no clarification) obtained poor removal rates of 10 to 75%, depending on phytoplankton species (Mouchet & Bonnelye, 1998). Conventional rapid sand filtration (clarified) should theoretically be more active than direct filtration (Svrcek & Smith, 2004). Slow sand filtration has lower loading rates; but it's also effective for eliminating cell-bound microcystins from drinking water, because of the additional possibility of biodegradation of

extracellular toxins on or inside the filter bed (Grützmacher, Wessel, Klitzke, & Chorus, 2010). As always an important concern during the process of filtration, death and lysis of cyanobacterial cells retained on filters could lead to toxins release (Chorus & Bartram, 1999).

APPENDIX 4: Chlorination of *Microcystis aeruginosa*: toxin release and oxidation, cellular chlorine demend and disinfection by-products formation

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Abstract:

Direct chlorination of toxic cyanobacteria cells can occur at various stages of treatment. The objectives of this work are to determine and model the extent of *Microcystis aeruginosa* cells lysis, toxins and organic compounds release and oxidation, and quantify the subsequent disinfection by-products formation. Chlorine exposure (CT) values of 110.7 and 100 mg.min/L were required to obtain 76% cell lysis and oxidation of released cell bound toxins at levels below the provisional World Health Organisation guideline value(1 µg/L MC-LR). Toxin oxidation rates were similar or faster than cell lysis rates in ultrapure water. This work presents much needed unit *Microcystis aeruginosa* cellular chlorine demand (5.6±0.2 pgCl_2/cell) which could be used to adjust the chlorination capacity to satisfy the total chlorine demand associated with the presence of cells. Furthermore, a novel successive reaction kinetics model is developed using the kinetics of the chlorine reaction with cyanobacterial cells and cell-bound toxins. Chlorination of dense cell suspensions (500,000 cells/mL) in ultrapure water at CT up to 3051 mg.min/L resulted in modest concentrations of trihalomethanes (13 µg/L) and haloacetic acids (below detection limit).

Keywords: Cyanobacteria, cyanotoxins, chlorination kinetics, chlorine demand, disinfection by-products

1. Introduction

Toxic cyanobacteria have been increasingly detected in drinking water sources and treated water (Lahti et al. 2001; Zamyadi, MacLeod et al. 2012). Microcystins (MCs) are a commonly found cyanotoxin with over 60 known analogous including Microcystin-LR (MC-LR) (Fischer et al. 2001; Svrcek and Smith 2004; Merel et al. 2009) (Supporting information Table SI-S1, Figure SI-S2 and Table SI-S3). *Microcystis aeruginosa* is one of the main cyanobacteria species producing these hepatotoxins and freshwater *M. aeruginosa* proliferation events have been observed worldwide (Svrcek and Smith 2004). The concentration of MCs in drinking water is regulated by different water authorities across the globe (Newcombe et al. 2010) including provisional World Health Organization (WHO) guideline limit of 1 µg/L of MC-LR for safe drinking water (Chorus and Bartram 1999). WHO recommends that in water containing MCs producing cyanobacteria, the MC-LR guideline value should be applied to the total cell-bound and extracellular MCs.

Recent intensive monitoring of cyanobacterial cells inside drinking water treatment plants (DWTPs) in Quebec (Canada) has evidenced the breakthrough and accumulation of toxic cells, with a dominance of MCs producing *M. aeruginosa* (McQuaid et al. 2011; Zamyadi, MacLeod et al. 2012; Zamyadi, McQuaid et al. 2012). Release of cyanotoxins can occur during conventional clarification by sedimentation and filtration over sand/anthracite processes and post-oxidation, most often chlorination, constitute an essential barrier against dissolved and cell-bound cyanotoxins to prevent cell and toxin breakthrough into drinking water (Pietsch et al. 2002; Schmidt et al. 2002; Schmidt and Konopka 2009; Schmidt, Petzoldt et al. 2009; Janjaroen et al. 2010; Zamyadi, MacLeod et al. 2012). Furthermore, direct chlorination of raw water or inter-chlorination are still practiced in many North American water treatment plants. If (pre-, inter- and/or post-) chlorination is conducted on water containing significant concentrations of cyanobacteria, it can lead to several water quality problems, e.g. cell damage and the subsequent release of cell-bound toxins, increased chlorine demand, dissolved organic carbon (DOC) release and elevated chlorination by-products (DBP) formation (Merel et al. 2010; Zamyadi et al. 2010). However, it may constitute a viable temporary treatment option if it is proven to be effective for removal of cell-bound and dissolved toxins, while not adversely affecting other water quality parameters. Therefore, operators of DWTP need to understand the limitations of pre-and post chlorination of *M. aeruginosa* cells and their associated toxins.

Chlorination is highly efficient for the oxidation of major dissolved MCs analogues under various water quality conditions and its kinetics can be predicted (Acero et al. 2005; Ho, et al. 2006; Acero et al. 2008; Merel et al. 2010; Zamyadi et al. 2012). Chlorine concentration and the contact time are the key factors in the confident application of chlorine for oxidation. The appropriate degree of oxidation is obtained using the concept of concentration multiplied by time, chlorine exposure, or CT. The CT value is calculated by determining the area under a graph of chlorine concentration versus time (Ho et al. 2006). The degree of cell-bound and extracellular MCs chlorination can be predicted by quantifying the rates of cell lysis, MCs release, and MCs oxidation under given water quality conditions (Daly et al. 2007).

Early laboratory testing suggested that doses required to maintain a residual in the distribution system (0.5 mg/L after 30 minutes of contact) at water temperatures ~20 ℃ with pH values under 8, resulted in the oxidation of total MCs below detection limits (Newcombe and Nicholson 2004). Chlorination was also found to be highly efficient to oxidize *M. aeruginosa* cells and subsequently cell-bound MCs using elevated concentrations of laboratory cultured cells (3×10^5–1×10^6 cells/mL) in natural water. Although cell bound toxin release was generally rapid and overall total toxin removal exceeded 90%, residual toxin concentrations ranging from 0.5 to 33 µg/L remained after CT exposures varying from ~0.7 to ~33.4 mg.min/L Cl^2 (Daly et al. 2007; Merel et al. 2010). More recently, the cell integrity of high concentrations (1.9×10^5–4.3×10^6 cells/mL) of laboratory grown cyanobacteria and natural blooms subjected to chlorination was investigated (Lin et al. 2009). These results suggest that laboratory grown *M. aeruginosa* in natural water may be more resistant to cell damage by chlorination than *Anabaena circinalis* and that small *M. aeruginosa* colonies from natural blooms were more resistant than single cell suspensions from laboratory cultures. Finally these authors also report limited oxidation of the low concentration of dissolved and cell-bound MC-LR_{equi} from a natural bloom.

Most laboratory based results converge in support of a predictable 92 rapid loss of cell integrity and subsequent release and oxidation of dissolved and cell-bound toxins that can be described by first-order apparent rate constants with respect to chlorine exposure. The toxin oxidation rate constants calculated from spiked dissolved conditions in ultrapure water are significantly higher (2-24×) than those estimated from the chlorination of released cell-bound toxins from cells suspensions (Daly et al. 2007). More modest differences (1.2-1.5×) in rates have been reported using lower cell densities of *Anabaena circinalis* for cell bound saxitoxin and of *M.*

*aeruginosa*for cell-bound MCs (Zamyadi et al. 2010; Zamyadi, Ho et al. 2012). However, the efficacy of the oxidation of released cell-bound toxins from natural blooms has been recently questioned in light of limited reports showing higher cyanobacteria cell resistance and poor oxidation of dissolved toxins in the presence of damaged cells (Zamyadi, MacLeod et al. 2012). Furthermore, prudent operation of the chlorination process in DWTP requires the knowledge of *M. aeruginosa* cellular chlorine demand, which is currently undetermined. This information is essential to ensure that sufficient chlorine capacity is present during peak cell concentration events.

It has been suggested that the chlorine demand of cyanobacteria cell-bound compounds and the consequent DBP formation potentials varies (Bond et al. 2009). Release of cell-bound compounds due to autolysis of cells is classified as extracellular organic matter (EOM) while those released from compromised cells is categorized as internal organic matter (IOM) (Fang et al. 2011). EOM and/or IOM could contribute to the formation of DBPs, e.g. trihalomethanes (THM) and haloacetic acids (HAA), due to the reaction of released material with chlorine (Hureiki al. 1994; Bond et al. 2009). Published results on the DBP formation potentials of IOM and EOM from cellular suspensions under chlorination are contradictory, reflecting differences in testing conditions especially chlorine dosage, growth phase, species and culture conditions. Significant DBP formation (e.g. total THM yields of 67-269 μg/L) has been reported in connection to reactive carbon associated with high cell numbers and their EOM (Graham et al. 1998; Huang et al. 2009; Zamyadi, Ho et al. 2012). However, chlorination of approximately 46,000 cells/mL of *Anabaena circinalis* cells in natural water (complete cell damage and oxidation of produced saxitoxins to below detection limit) with a CT of 50.3 mg.min/L only caused a modest increase of 9 μg/L of total THM (TTHM) and 3 μg/L of HAA9 compared to the control assay (chlorination of natural water without cyanobacteria cells) (Zamyadi et al. 2010).

Systematic studies are needed to better quantify the chlorine demand per cell and DBP formation associated with cell materials in conditions representative of DWTP operation. The objectives of this research were: (1) to determine and to model the *M. aeruginosa* cells lysis, toxins and DOC release, and MCs oxidation during chlorination of ultrapure and natural water spiked with laboratory cultures; (2) to estimate the *M. aeruginosa* cellular chlorine demand when cell lysis occur due to chlorination; and (3) to study the DBP formation associated with the chlorination of *M. aeruginosa* cell-bound materials.

2. Material and Methods

2.1. Materials and Reagents

A toxic strain of M. aeruginosa, isolated from Pretzlaff Pond, Alberta, Canada, was cultured in the laboratory in artificial seawater medium (ASM-1 – Table SI-S4) (Gorhamet al. 1964). ASM-1 media (20 L) was sterilized at 120oC, and then after pH adjustment, inoculated with an ASM-1 grown culture in stationary phase. The culture was incubated at 26oC under 6 hours rotating light-darkness flux at light intensity of 70 μmol s-1 m-2. All chemicals and reagents used for analytical methods calibration were laboratory analytical grade from various suppliers.

2.2. Water Source and Quality

Water was sourced from the Mille-Îles River (MIR) in Quebec, Canada. Samples were collected from the St-Rose DWTP intake prior to any treatment. Untreated MIR water was filtered over pre-rinsed Binder-free glass microfiber 1.2 μm filter (GF/C - Whatman, UK) prior to inoculation with the culture. The water had a DOC concentration of 6.3 mg/L, UV absorbance (at 254 nm) of 0.191 cm-1, specific UV absorbance of 3.0 L/mg/m, pH of 7.4, and an alkalinity of 19 mg/L as CaCO3. Ultrapure water (Millipore Pty Ltd, USA) was also used for selected experiments. Prior to DOC analyses, samples were passed through pre-rinsed (1L ultrapure water) 0.45μm cellulose nitrate Supor®-450 membrane filters (PALL Life Sciences, USA). DOC measurements were made using a 5310C total organic carbon analyzer (Sievers Instruments Inc., USA). pH was measuredusing a Fisher Scientific pH meter (Accumet, Fisher Scientific Instruments, USA) which was calibrated with pH 4, 7 and 10 standard buffers (Fisher, USA).

2.3. Chlorination experiments

Chlorine stock and chlorine residual concentrations were determined using the DPD-FAS titration method described in Standard Methods (American Public Health Association Three separate chlorination experiments were conducted as: (**1st Cl_2 Exp**) MCs producing *M. aeruginosa* suspensions in ultrapure water; (**2nd Cl_2 Exp**) *M. aeruginosa* suspensions in MIR water; and (**3rd Cl_2 Exp**) cell-bound *M. aeruginosa* cellular material in ultrapure water. Each chlorination experiment was preceded by a chlorine decay experiment. For chlorine decay experiments, water samples were dosed with desired chlorine concentration and triplicate samples were taken at specified contact times for free chlorine residual determinations using the

titration method. All chlorination experiments were conducted in triplicate in brown amber bottles at room temperature (20±2 ℃).

Cultured *M. aeruginosa* cells were enumerated on a compound microscope in a Sedgewick-Rafter counting chamber after preservation in Lugol's iodine (Cooperative Research Centre for Freshwater Ecology (CRCFE) 1999). Required volume of stationary phase culture to be spiked in ultrapure and MIR water to achieve desired cell suspension was adjusted based on the culture cell number. The number of cells in water samples after inoculation was confirmed by microscopic enumeration. Detection of cell auto fluorescence using fluorescence microscopy was used to assess cell integrity. Healthy cells will emit bright red fluorescence when irradiated with green excitation light (Nancharaih et al. 2007). Control (non-chlorinated) water samples showed no sign of cell integrity loss due to direct addition of cells to ultrapure and MIR water (data not shown) similar to results from Zamyadi et al. (2010). All chlorination experiments regardless of water quality parameters were conducted with a chlorine residual after 60 minutes of contact.

1st Cl_2 Exp: These experiments were conducted in ultrapure water buffered 183 at pH 8 with phosphate (0.01M) (American Public Health Association 2005). Chlorine was added from the chlorine stock solution to obtain the Cl_2 doses of 2, 3 and 4.5 mg/L for *M. aeruginosa* suspensions of 60,000 (±2%), 250,000 (±4%) and 500,000 (±4%) cells/mL, respectively. Samples were quenched at specified contact times with sodium thiosulphate at a stoichiometric ratio specified in Standard Methods (American Public Health Association 2005). Quenched samples were taken for DOC measurement. To determine the cell lysis due to chlorination total cell counts (microscopic counts in triplicate) were conducted on 2 mL samples preserved with Lugol's iodine (Cooperative Research Centre for Freshwater Ecology (CRCFE) 1999). Remaining quenched water samples were divided in two subsamples of 250mL: the first subsample was immediately filtered through 0.45 μm filter (Whatman, UK) to remove cellular material, and then analyzed for extracellular MCs. The second subsample was subjected to three freeze–thaw cycles before filtration over 0.45 μm filters to analyze for total MCs (both the cell-bound and extracellular compounds). During each freeze-thaw cycle, samples were frozen at -25 and then they were thawed by exposing the well sealed sample containers to 5^{o}C water. Samples were always kept in the dark. Pre-tests were conducted to confirm the efficiency of freeze-thaw procedure to release cell-bound toxins. The entire cell-bound toxins were released after the second cycle and the third cycle was conducted to be on the safe side. For all MCs analysis,

confidence limits were calculated using the standard deviations of triplicate measurements. For all experiments the extracellular concentrations of MCs was always within or below the confidence limit of total concentration. The difference between total and extracellular levels of MCs was estimated as cell-bound.

2nd Cl_2 Exp: These experiments were conducted in MIR water with phosphate buffer (0.01M) controlled pH values of 7 and 8.5. Chlorine was added from the chlorine stock solution to obtain the Cl_2 doses of 2, 5 and 10 mg/L for *M. aeruginosa* suspensions of 50,000 (±2%) cells/mL. Similar to "1st Cl_2 Exp" quenched samples were taken for DOC measurement and cell lysis determination. Cell densities (50,000 cells/mL corresponding to 0.12 mg/L DOC) were constant (±2%) and background MIR water DOC varied by less than 10%.

3rd Cl_2 Exp: These experiments were conducted on *M. aeruginosa* suspension of 500,000cells/mL in ultrapure water buffered at pH 8 with phosphate buffer (0.01M). Cultured cells were filtered and re-suspended in ultrapure water, thus eliminating the contribution of EOM. No sign of cell integrity loss was observed due to separation of cells from the culture media (data not shown). The *M. aeruginosa* suspension was subjected to three freeze–thaw cycles for complete release of intracellular material before chlorination (McQuaid et al. 2011). Chlorine was added from the chlorine stock solution to obtain the Cl_2 dose of 5.5 mg/L. Residual chlorine concentrations were determined after 30 and 1440 minutes of contact time and then samples were quenched (by sodium thiosulphate) at a stoichiometric ratio (specified in Standard Methods). Quenched samples were taken for DOC and DBP measurements.

2.4. Analysis of MCs and DBPs

MCs analyses were conducted using an Abraxis Microcystins ADDA ELISA Plate (an enzyme-linked immunosorbent assay – Abraxis LLC, Pennsylvania, USA). The analyses of MCs concentrations were measured as μg/L of MC-LR equivalent (MC-LR eq.). The accuracy of the plate was validated using standard toxin materials (229 Zamyadi, MacLeod et al. 2012).

Samples taken for DBP detection were analyzed for four THMs (bromoform, chloroform, bromodichloromethane, and dibromochloromethane) as total THM (TTHM) and six HAA (bromoacetic acid, bromochloroacetic acid, chloroacetic acid, dibromoacetic acid, dichloroacetic acid, and trichloroacetic acid) as HAA6. THMs were extracted by liquid/liquid extraction with hexane as described in Standard Methods 6232B (American Public Health Association 2005).

HAA compounds were extracted by liquid/liquid extraction with methyl tert-butyl ether (MtBE) followed by derivatization with acidic methanol in accordance with USEPA Method 552.2 (United States Environmental Protection Agency (USEPA) 1995). All DPBs were analyzed by gas chromatograpy (CP-3800, Varian) equipped with an electron capture detector (GC/ECD).

2.5. Data analysis

Reactions between chlorine and the *M. aeruginosa* cells and MCs are of second order but can be described by a first order process with respect to chorine exposure (Daly et al. 2007; Lin et al. 2009; Zamyadi, MacLeod et al. 2012). In this study, cell lysis is defined as cell damage sufficient to render them undetectable under the microscope in a preserved sample (i.e. loss of cell count). Equation 1 was used to calculate the cell lysis or toxin degradation rates for these chlorination experiments (Daly et al. 2007; Lin et al.2009):

$$Ln\left(\frac{[X_{CT}]}{[X_0]}\right) = -k \times CT \qquad \text{Equation 1}$$

where CT equals the chlorine exposure; X_{CT} equals the number of lysed 251 cells or MC-LReq. concentration after a given chlorine exposure; X_0 equals the number of lysed cells or MC-LR eq. concentration at CT=0; and *k* equals the rate at which *M. aeruginosa* cells (k_{lysis}) or MC-LR eq (k_{appt}) oxidation occurs (Daly et al. 2007; Lin et al. 2009).

The definitions of chlorination rates (*k*) used in this study are as follows:

- k_{lysis}: rate of total *M. aeruginosa* cell destruction
- $k_{release}$: rate of loss of *M. aeruginosa* cell integrity
- $k_{total\ MC\text{-}LR}$: rate of oxidation of total MC-LR including both cell-bound and dissolved toxins
- $k_{dissolved\ MC\text{-}LR}$: rate of oxidation of only dissolved MC-LR whether in studies where standard MC-LR extracts were spiked in water or where entire cell-bound toxins are released into water.

3. Results and discussion

3.1. Impact of *M. aeruginosa* cellular compounds and water matrix on chlorine demand

Free chlorine decay and cell lysis experiments were conducted within **1st Cl_2 Exp** and **2nd Cl_2 Exp** (Figure 1). Standard deviation of these cell enumerations (triplicate enumeration/sample) fit within the confidence limit of cell count method (Lund et al.1958; Laslett et al. 1997; Hurst et al. 2007) (data not shown). Chlorine demand in ultrapure water is directly related to the *M. aeruginosa* cell numbers (Figure 1a). The chlorine demand in MIR water in the absence of cells is lower than in the presence of cells with similar trends for both pH values (Figures 1b & 1c).

Chlorine decay data were used to calculate the CT values by fitting 274 a two pathway integration model to a two phase decay curve: fast (<1 min) and slow (Daly et al. 2007; Zamyadi et al. 2010). Table 1 presents the resulting first order rate constants for chlorine decay. The slow apparent rate constants (k_{Cl2}) in ultrapure water without cells are very stable at all pH values (Table 1). The addition of cyanobacteria cells to the ultrapure water augments the apparent rate constant values (Table 1). Therefore, indicating the introduction of more natural organic matter (NOM) to water sample which are reactive to chlorine. Also, the k_{Cl2} values increased with increases in cell numbers and chlorine doses. Greater cell numbers were associated with higher DOC release (See section 3.4) consistent with the observed faster chlorine decay. In natural MIR water the apparent rate constants are similar at pH 7 and 8.5 for each chlorine dose, but increase with the addition of cells and higher chlorine doses (Table 1).

Table 1. First-order apparent rate constants (k_{Cl2}) in s^{-1} for chlorine decay. All correlation coefficients (R^2) exceeded 0.85.

Type of water	Cell nb. Cells/mL	2 mg/L Cl_2		3 mg/L Cl_2		4.5 mg/L Cl_2	
		pH 8		pH 8		pH 8	
Ultrapure	No cells	1.7×10^{-5}		1.6×10^{-5}		1.7×10^{-5}	
	60,000	6.4×10^{-5}		-		-	
	250,000	-		9.7×10^{-5}		-	
	500,000	-		-		1.5×10^{-4}	
	Cell nb. Cells/mL	2 mg/L Cl_2		5 mg/L Cl_2		10 mg/L Cl_2	
		pH 7	pH 8.5	pH 7	pH 8.5	pH 7	pH 8.5
MIR	No cells	8.4×10^{-5}	8.1×10^{-5}	1.8×10^{-4}	1.8×10^{-4}	1.1×10^{-3}	1.1×10^{-3}
	50,000	8.4×10^{-5}	8.2×10^{-5}	1.9×10^{-4}	1.9×10^{-4}	1.9×10^{-3}	1.9×10^{-3}

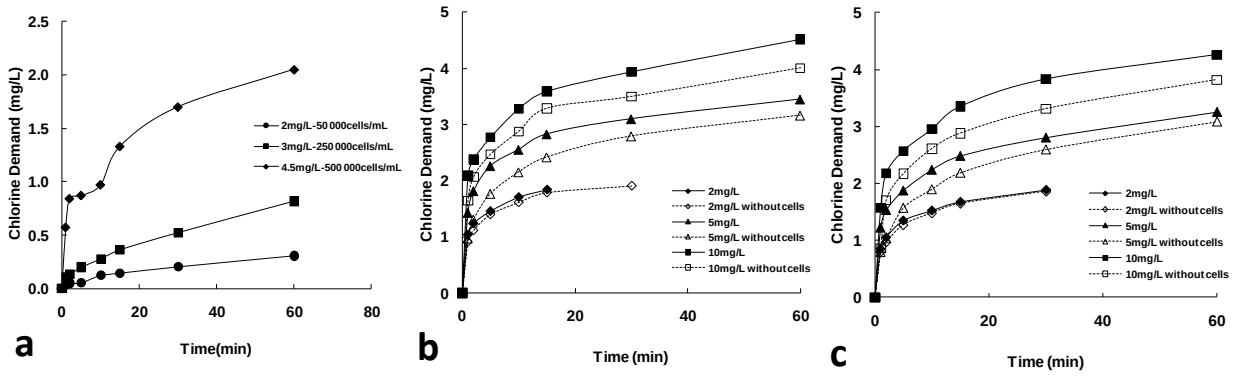

Figure 1. Chlorine demand of *M. aeruginosa* suspensions in (a) ultrapure water with 60,000, 250,000 and 500,000 cells/mL under 2, 3 and 4.5 mg/L Cl_2 respectively at pH 8, and 50,000 cell/mL under 2, 5 and 10 mg/L Cl_2 (b) at pH 7 and (c) pH 8.5.

3.2. Chlorination effect on *M. aeruginosa* cells lysis and estimation of chlorine demand per cell

The response of three *M. aeruginosa* cell suspensions (60,000±2%, 250,000±4% and 500,000±4% cells/mL) in ultrapure water (pH 8) to different chlorine doses (respectively 2, 3 and 4.5 mg/L) was evaluated in **1st Cl_2 Exp**. Figure 2a (**1st Cl_2 Exp)** demonstrates that chlorination in ultrapure water spiked with highest cell number caused 76% of cell lysis after 60 minutes of contact (CT = 130 mg.min/L). Additionally, the response of the *M. aeruginosa* cell suspension (50,000±2% cells/mL) in MIR water (pH 7 and 8.5) to 2, 5 and 10 mg/L of Cl_2 was studied (**2nd Cl_2 Exp**). Figure 2b (**2nd Cl_2 Exp**) shows that similar levels of cell lysis were achieved with the same or higher CT values in MIR water.

Zamyadi et al. (2010) observed a much lower (15%) reduction in total cell numbers (lysis) with a CT of ~50 mg.min/L in a suspension of 46,000 cell/mL of *Anabaena circinalis*. However, they also reported that over 98% of cells (even detectable under microscope in preserved samples) could be categorized as injured/dead cells (damaged membrane or metabolically inactive cells) at a CT of less than 7 mg.min/L. It is important to consider that cell lysis, here defined as corresponding to the loss of detectable stained cell structures, is a less conservative indicator of loss of viability as it occurs at the last step of cell decay.

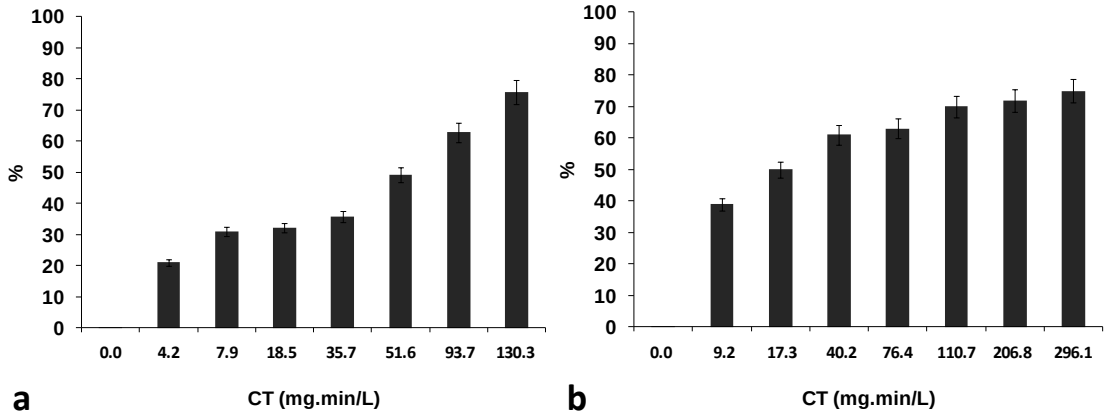

Figure 2. Reduction in number of cells (cell lysis) during chlorination of *M. aeruginosa* suspensions of (a) 500,000 cells/mL in ultrapure water at pH 8 with 4.5 mg/L Cl_2 (similar results were observed in other chlorination experiments in ultrapure water) and (b) 50,000 cells/mL in MIR water at pH 8.5 with 10 mg/L Cl_2 (similar results were observed in other chlorination experiments in MIR water).

The assumption of a first-order reaction with respect to chlorine reaction and the *M. aeruginosa* cells lysis is feasible (Daly et al. 2007; Lin et al. 2009) and the rate of cell lysis (k_{lysis}) can be described using Equation 1. The k_{lysis} calculated for chlorination of *M. aeruginosa* cells are presented in Table 2. Similar k_{lysis} values were observed in both experiments conducted in ultrapure and natural water.

The k_{lysis} values in MIR water are close to chlorination lysis rates estimated for laboratory cultures of *M. aeruginosa* (Zamyadi et al. 2012) and in natural bloom samples (Zamyadi, MacLeod et al. 2012) (Table 2). In other studies, the rates of cell lysis after chlorination ($k_{release}$) was determined by cell integrity tests rather than loss of cell count and so may not be directly comparable. $k_{release}$ represents the release of cell bound material even if the cells are still present in water. $k_{release}$ from cultured cells in chlorination assays have been found to be higher compared to those measured for cells from natural blooms (Lin et al. 2009; Zamyadi et al. 2012). In other cases (Ding et al. 2010), $k_{release}$ values for cultured cells fall within the range of k_{lysis} values estimated in this work. These differences in susceptibility can be attributed to the physiological state of the *M. aeruginosa* cells, the cell densities, the water matrix and the chlorination conditions (Pietsch 320 et al. 2002; Lin et al. 2009).

M. aeruginosa cellular chlorine demand per cell can be estimated from the lysis of *M. aeruginosa* suspensions in ultrapure water in the presence of chlorine residual (Figures 1 and 2a). The cumulative chlorine demand after 60 minutes of contact per number of lysed cells is similar (5.6±0.2 pgCl_2/cell) for each of the three experiments conducted in ultrapure water, suggesting that cell numbers can be used to estimate the cellular chorine demand. This novel information of unit cellular chlorine demand can help DWTP operators estimate if their chlorination capacity is sufficient to satisfy the total chlorine demand associated with the presence of cyanobacteria. However, since these estimations were obtained on unicellular laboratory cultures, validation of these observations in the presence of cyanobacterial colonies appears desirable.

Table 2. The apparent rate constants for cell lysis or reduction in total cell count (k_{lysis} - $M^{-1}s^{-1}$), for loss of cell integrity or cell rupture ($k_{release}$ - $M^{-1}s^{-1}$), for total toxins oxidation (k_{total} - $M^{-1}s^{-1}$) and for the oxidation of dissolved toxins released from damaged cells ($k_{dissolved}$ - $M^{-1}s^{-1}$) derived from this study compared to published results. Comments in parenthesis describe the origin of the CB cells (from laboratory cultures or environmental bloom samples) and the toxins (from standard extracts or cell-bound). * MC-LR equivalent

	This study									Published studies							
	60,000 cells/mL, Ultrapure water, 2 mg/L Cl_2, pH 8	250,000 cells/mL, Ultrapure water, 3 mg/L Cl_2, pH 8	500,000 cells/mL, Ultrapure water, 4.5 mg/L Cl_2, pH 8	50,000 cells/mL, MIR water, 2 mg/L Cl_2, pH 7	50,000 cells/mL, MIR water, 2 mg/L Cl_2, pH 8.5	50,000 cells/mL, MIR water, 5 mg/L Cl_2, pH 7	50,000 cells/mL, MIR water, 5 mg/L Cl_2, pH 8.5	50,000 cells/mL, MIR water, 10 mg/L Cl_2, pH 7	50,000 cells/mL, MIR water, 10 mg/L Cl_2, pH 8.5	Acero et al. 2005. pH 7.9-8	Ho et al. 2006. pH 7.9	Rodriguez, Onstad et al. 2007. pH 8	Daly et al. 2007. pH 6.8-7.6	Lin et al. 2009. pH 8.3-8.6	Ding et al. 2010. pH 7.6	Zamyadi, Ho et al. 2012. pH 8	Zamyadi, MacLeod et al. 2012. pH 8.3
M. aeruginosa k_{lysis} ($M^{-1}s^{-1}$)	27.9	17.9	11.6	234.1	170.2	73.1	33.3	30.6	11.5	-	-	-	-	-	-	37 (culture)	3.8-16* (bloom)
M. aeruginosa $k_{release}$ ($M^{-1}s^{-1}$)	-	-	-	-	-	-	-	-	-	-	-	-	670±77	790-1100 (culture), 70-590 (bloom)	43.6 (culture)	756-1030 (culture)	-
k_{total} MC-LR	33.5 *	49.6 *	88.7*	-	-	-	-	-	-	-	-	-	10-96 (cell-bound)	-	-	21-23.3 (cell-bound)	2.1-17.9* (bloom)
$k_{dissolved}$ MC-LR	-	-	20.2*	-	-	-	-	-	-	33.1-38.0	110 - 170	33	242	-	55.9	-	-

3.3. Impact of chlorination on cell-bound MCs release and oxidation and modeling

Figure 3 shows the release and oxidation of cell-bound MCs (MC-LR eq.) in ultrapure water for the *M. aeruginosa* suspensions of 60,000, 250,000 and 500,000 cells/mL. Complete toxin release and oxidation of toxins to below the WHO guideline value (1 μg/L MC-LR for DW) was observed with a CT of 100 mg.min/L (Figure 3). The observed toxins release and oxidation (Figure 3) also agree with cell lysis (Figure 2a). Kinetics models can express the chlorination of released cell-bound and dissolved MCs (Daly et al. 2007; Rodriguez et al. 2007; Lin et al. 2009). Rate constants (k_{total}) for the oxidation of total MC-LR eq. in ultrapure water can be derived using Equation 1. k_{total} represents the combined rate of the release of cell-bound toxins due to 342 cell lysis and the oxidation of the released toxins.

When using dense suspensions of 500,000 cells/mL of *M. aeruginosa* in ultrapure water (Figure 3c), it is possible to estimate the rate constant for the degradation of dissolved toxins ($k_{dissolved}$) after complete release as indicated by the elevated cell lysis. This estimate of $k_{dissolved}$ model may be considered as more representative of conditions of oxidation of dissolved toxins in the presence of cellular debris, as would be the case during a bloom.

The observed apparent rate constant $k_{dissolved}$ from this study and previously published rate values are listed in Table 2. Our estimate of $k_{dissolved}$ for MC-LR eq. is equivalent or lower than those published for spiked toxins in ultrapure and natural waters (Acero, et al. 2005; Rodriguez et al. 2008; Ding et al. 2010). The reported values of $k_{dissolved}$ vary by 7.3 times with the highest values observed in spiked natural water or in the presence of elevated densities of cells. Differences can be explained partly by slight variations in experimental pH as the oxidation of MC-LR is more efficient at pH values below 8 (Ho et al. 2006). This phenomenon has been attributed to the degree of MC-LR protonation at lower pH values making it more susceptible to chlorination (Ho et al. 2006). The impact of NOM can also be significant because of organic matter-MC interactions or the action of highly reactive quaternary chloramines on organic matter moieties (Ho et al. 2006).

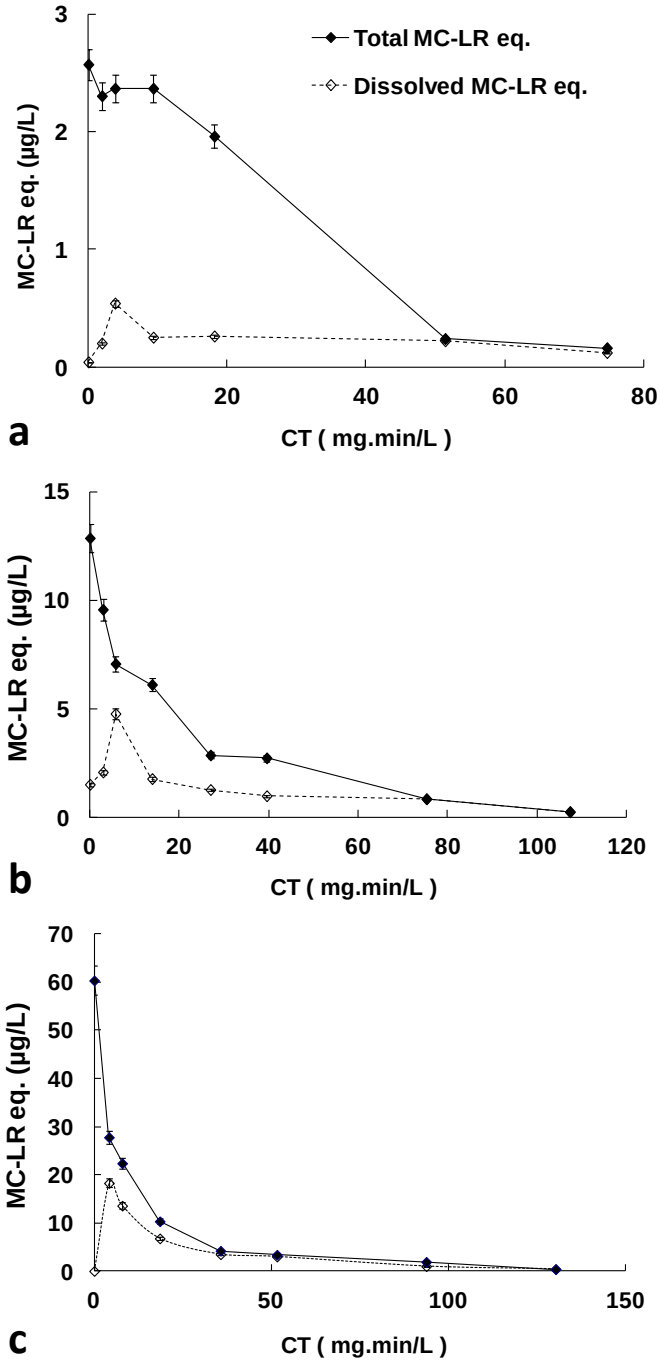

Figure 3. Toxins release and oxidation from *M. aeruginosa* suspensions in ultrapure water at pH 8 with cell number and chlorine doses of (a) 60,000 cells/mL and 2 mg/L Cl_2, (b) 250,000 cell/mL and 3 mg/L Cl_2, and (c) 500,000 cells/mL and 4.5 mg/L Cl_2, respectively.

Our experiments support previous observations of rapid oxidation of cell-bound toxins upon release from damaged or lysed cells. In most cases, chlorination causes a fast release of cell-bound toxins as shown by the $k_{release}$ values. Overall, the oxidation of toxins, whether total (k_{total}) or dissolved ($k_{dissolved}$) occurs somewhat more quickly than cell lysis (k_{lysis}), suggesting that released cell-bound toxins react faster 365 with chlorine than the remaining damaged cells and debris. The reported values are variable with notable differences and exceptions observed in some examples (Table 2). However, larger differences have been reported for MC-LW, MC-LF, MC-RR, MC-YR and MC-LA chlorination rates (3320, 204, 136, 94 and 89.5 $M^{-1}S^{-1}$ respectively) as compared to cell inactivation rate (43.6 $M^{-1}S^{-1}$) (Ding et al. 2010). Definition of cell lysis is different between publications resulting in varying comparison of toxin oxidation rates with cell lysis rates.

The loss of cell integrity (and by inference toxin release) and toxin degradation as a function of chlorine exposure (CT) can be described by first order processes and can be considered as consecutive reactions, as shown in Equation 2. In these reactions the cell-bound toxins (A in Equation 2) are first released by the action of chlorine on the cell and then the dissolved toxins (B in Equation 2) are degraded by chlorine (C in Equation 2). If this assumption is valid then, the concentration of cell-bound and dissolved toxin can be described by Equation 3 and Equation 4 respectively (Jones 1970).

$$A \xrightarrow{k_{release}} B \xrightarrow{k_{dissolved}} C \qquad \text{Equation 2}$$

$$A = A_0 e^{-k_{release} CT} \qquad \text{Equation 3}$$

$$B = B_0 e^{-k_{dissolved} CT} + \frac{A_0}{1 - k_{dissolved}/k_{release}} \left(e^{-k_{dissolved} CT} - e^{-k_{release} CT} \right) \qquad \text{Equation 4}$$

$$CT_{\max} = \frac{\ln\left(\frac{k_{dissolved}}{k_{release}} \left(1 + \frac{B_0}{A_0} \left(1 - \frac{k_{dissolved}}{k_{release}} \right) \right) \right)}{-(k_{release} - k_{dissolved})} \qquad \text{Equation 5}$$

where A_0 and A are the concentration of cell-bound toxin at CT = 0 and a given CT, respectively; B_0 and B are the concentration of dissolved toxin at CT = 0 and a given CT, respectively; C is the amount of toxin degraded at given CT; $k_{release}$ is the release rate of cell-bound toxin; $k_{dissolved}$ is the

degradation rate of dissolved toxin; CT is the chlorine exposure. If Equation 4 adequately describes the process then the Equation 5 could be used to determine the chlorine exposure (CT_{max}) that would yield the maximum concentration of free dissolved toxin i.e. the worst case scenario for drinking water treatment. Treatment plant operators would need to ensure contact with chlorine exceeded CT_{max} to achieve adequate degradation of toxin.

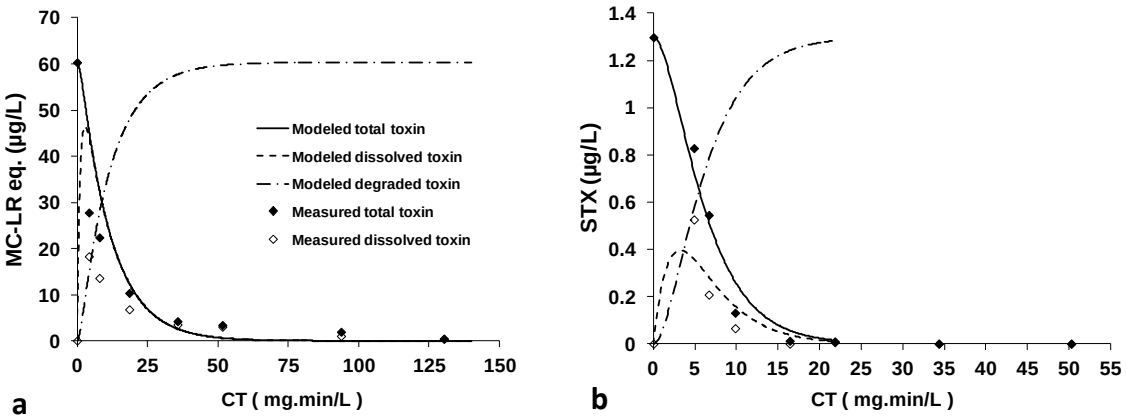

Figure 4. Comparison of model predicted toxin concentrations with (a) experiment results presented in Figure 3c (chlorination of 500,000 cells/mL of *M. aeruginosa* using 4.5 mg/L Cl_2 in ultrapure water at pH 8), and (b) experiment results on chlorination of 46,000 cells/mL of *Anabaena circinalis* and their associated saxitoxin using 3 mg/L Cl_2 at pH 8 presented by Zamyadi et al. 2010 (A. Zamyadi, et al., 2010), using experimentally determined toxins release and degradation rates.

The results of 1st Cl_2 Exp (Figure 3c) are modeled using the experimentally determined loss of cell integrity ($k_{release}$) and toxin degradation ($k_{dissolved}$) rates (Figure 4a). The $k_{release}$ for chlorination of *M. aeruginosa* cells was obtained from Zamyadi, Ho et al. (2012) presented in Table 2. The $k_{dissolved}$ (20.2 – Table 2) is estimated from the chlorination of dissolved MC-LR eq. released during oxidation of 500,000 cells/mL of *M. aeruginosa* in ultrapure water using 4.5 mg/L Cl_2 at pH 8. The model correctly describes the decrease of total MC-LR eq. concentrations (R_2 = 0.91) and the trends for dissolved toxins (Figure 4a). Data from the chlorination of toxic *Anabaena circinalis* and the published constants were used to further test the validity of the model (Zamyadi et al. 2012). Values of $k_{release}$ (1600 $M^{-1}S^{-1}$) and $k_{dissolved}$ (370.5 $M^{-1}S^{-1}$) were obtained using 3 mg/L of Cl_2 at pH 8 in natural water for 6,000 cells/mL of saxitoxin producing *A. circinalis*. Figure 4b shows again excellent predictions for the oxidation of total saxitoxin

(R_2=0.98) and good concordance for dissolved toxins. For both cyanobacteria species, the model outcome for the oxidation of total toxins fits better than for dissolved toxins, at the lower range of CT values. This difference in the oxidation of dissolved toxins could be explained by: (1) the impact of the presence of cells on the oxidation of dissolved toxins; and (2) the short term variation 410 in the release rates attributable to the physiological status of the cyanobacterial cells. Although general fit of the model show acceptable agreement, the variability in describing the sequential release and oxidation at very low CTs could be improved by additional intensive testing. However, the close agreement of predicted and measured values after a minimum CT of 10 mg.min/L show the applicability of this approach to estimate operational boundaries for chlorination.

3.4. Chlorination effect on cellular organic carbon release and DBP formation

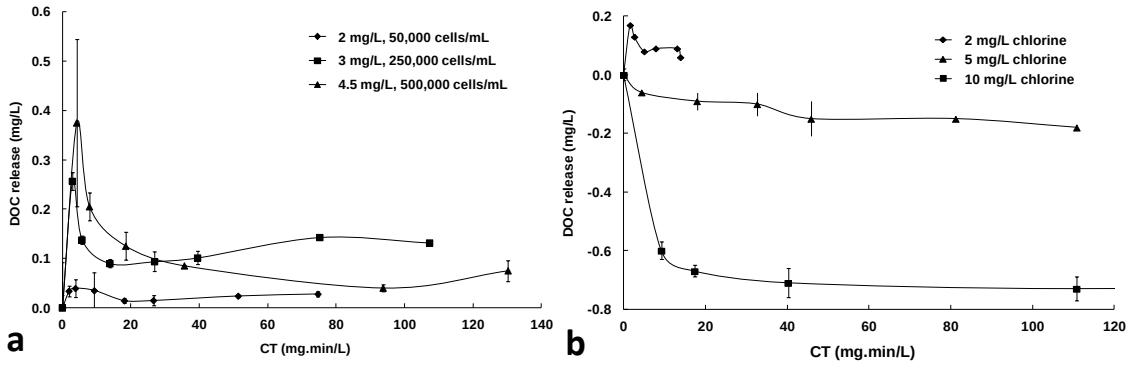

Figure 5. DOC release from oxidation of *M. aeruginosa* suspensions in (a) ultrapure water at pH 8 and (b) MIR water at pH 8.5 spiked with 50,000 cells/mL (results in MIR water at pH 7 were similar to those of pH 8.5).

Figure 5 demonstrates the effect of chlorination on DOC in increasing concentrations of *M. aeruginosa* suspensions in ultrapure and MIR water at pH 8.5. The final DOC of cellular suspensions in ultrapure water increased after 60 minutes of chlorination compared to their DOC prior to chlorination (Figure 5a). The maximum DOC release occurred quickly within the first quenching time (1 minute with a maximum CT of 4.2 mg.min/L for chlorination of 500,000 cells/mL with 4.5 mg/L of Cl_2). This release corresponds to a fraction (<10%) of the total carbon cell content estimated at 1.1 mg/L for 50,000 cells/mL equivalent (Zamyadi, Ho et al. 2012), and the maximum released concentrations reflect the increasing concentrations of cells added. The release is immediately followed by a rapid decrease in DOC concentrations (Figure 5a) that is

most probably attributable to the flocculent action of the hydrophilic polysaccharides released from the damaged cells (Henderson, Baker et al. 2008).

DOC variations after chlorination of *M. aeruginosa* suspension (50,000 cells/mL) in MIR water with 2 mg/L of Cl_2 follow a similar trend to that observed in ultrapure experiments Figure 5b). In contrast, this trend is greatly enhanced when applying a higher dosage of chlorine (5 and 10 mg/L) to the cellular suspensions (50,000 cells/mL) in natural water. In that case, a net decrease of DOC up to 0.73 mg/L C is observed from the background DOC values of 6.3 mg/L of the MIR water (Figure 5b). Similar trends were observed at a buffered pH of 7 (data not shown). This further substantiates the hypothesis that increasing dosages favors the formation of polysaccharides based compounds that transform algogenic organic matter (AOM) and humic acids from the natural water to particulate NOM.

The potential contribution of cyanobacterial cells to the formation of chlorination by products has been quantified as minor compared with that of background NOM in surface water (Zamyadi, Ho et al. 2012). However, DBP yields have shown that cyanobacterial cell-bound DOC can be a significant source of DBP precursors (Wardlaw et al. 1991; Plummer and Edzwald 2001; Nguyen et al. 2005; Huang et al. 2009) and more recent evidence shows that carbohydrates can contribute to the long term (72-400h) formation of THMs in the presence of bromide and 10 mg/L Cl_2 (Navalon et al. 2008).

The third chlorination experiment (3^{rd} Cl_2 Exp) was conducted to further investigate the reactivity of cyanobacterial organic matter, whether dissolved or particulate and specifically to investigate the contribution of *M. aeruginosa* cells to DBP formation during chlorination. The cells were filtered and re-suspended in ultrapure water, thus eliminating the contribution of EOM which can vary broadly depending on the cell growth phase and culture conditions, and can reach up to of 40 mg/L C in extremely dense suspensions (Hoyer et al. 1985; Nguyen et al. 2005). A very dense suspension of 500,000 cells/mL was subjected to three freeze–thaw cycles to release internal organic matter prior to chlorination with 5.5 mg/L Cl_2 at pH 8. Table 3 presents 456 the TTHM and HAA6 formation in the compromised *M. aeruginosa* suspension after 24 hours. The measured concentrations of TTHM and HAA6 after the maximum CT of 3051.2 mg.min/L still fall well below the current reference standard of 80 μg/L for TTHM and 60 μg/L for HAA5 (United States Environmental Protection Agency (USEPA) 2006).

Table 3. DBP formation in chlorination of lysed *M. aeruginosa* suspension (DOC of 1.30) of 500,000 cells/mL in ultrapure water at pH 8.

Time	Cl_2 residual	Cl_2 demand	CT	TTHM	HAA6
Min	mg/L	mg/L	mg.min/L	µg/L	µg/L
0	5.5	-	0.00	BDL*	BDL
30	2.9	2.6	125.4	BDL	BDL
1440	1.3	4.2	3051.2	12.87	BDL

* Below Detection Limits (BDL)

The results of this study contrast those from Huang et al. (2009) who reported high yields of THM and HAA precursors from high density suspensions of cultured *Microcystis* sp. (high DOC) during a chlorination experiments (pH 7 with over 0.5 mg/L Cl_2 residual from 1 to 7 days of contact). The measured DOC of the suspension of 500,000 cells/mL of *M. aeruginosa* (suspension with highest cell number and completely released cell bound material) is 1.3 mg/L. The highest published chloroform yield for chlorination of *Microcystis* sp. is 61 µg $CHCl_3$/mg C (Huang et al. 2009). Hence, 39.7µg/L is the maximum potential THM formation for the *M. aeruginosa* suspension of 500,000 cells/mL. Actual THM yield from the chlorination of IOM and EOM of this cell suspension within a contact time of 24 hours (Table 3) is below the maximum THM potential formation values. The published maximum potential yield of HAA precursor for concentrated *Microcystis* sp. culture is smaller than the published values for chloroform (Huang et al. 2009). However, the actual HAA6 measured in chlorination of total organic matter of 500,000 cells/mL of *M. aeruginosa* after 24 hours was below detection limit (Table 3).

The *M. aeruginosa* cells used for this study were harvested only at stationary phase of growth. Types of protein and carbohydrate associated with cyanobacterial cells vary with their growth phase (Henderson, Parsons et al. 2008; Henderson, Baker et al. 2008) and may vary the reactivity of these compounds with chlorine and their DBP formation potentials. During the exponential growth phase of cyanobacteria cells low molecular weight intermediate products (e.g. glycolic

and amino acids) with high reactivity to chlorine are dominant in cellular organic matter, while in aged cells (late stationary phase) high molecular weight products (e.g. polysaccharides) are abundant (Nguyen et al. 2005; Huang et al. 2009).

4. Conclusion

Results of this research demonstrate that chlorine doses of 2 to 10 mg/L cause the rapid release and subsequent oxidation of dissolved and cell-bound MCs from *M. aeruginosa*. Additionally, a maximum 76% reduction in total cell numbers (cell lysis) was observed with a CT of 296 mg.min/L. Kinetic analysis of cell-bound MCs oxidation in the presence of cellular debris highlights the differences with previous studies conducted using only dissolved toxin materials. These data provide novel information on chlorine demand per *M. aeruginosa* cell to obtain cell lysis and toxin release.

The cellular chlorine demand in addition to the toxin chlorination kinetics in the presence of cellular debris are key parameters to allow the operators of DWTPs to adjust the chlorination practice to maximize cell lysis and toxin oxidation. It is possible to model the fate of total toxins during chlorination of toxic cyanobacterial cells using both cells and toxin chlorination kinetics. This paper provides a simple model to predict the release of cell-bound toxins by the action of chlorine on the cells, and the subsequent degradation of dissolved toxins by chlorine. This information is relevant to water authorities and DWTPs operators to validate the efficiency of chlorination barrier. It can be used to assess potential responses to toxic cyanobacterial breakthrough events based on historic and climate change scenarios.

Our results show that chlorination of 500,000 cells/mL and less of *M. aeruginosa* cells do not contribute significantly to the pool of DBP precursors. However, the background NOM and its DBP formation potential will determine whether the chlorination of *M. aeruginosa* cells can be considered without the formation of excessive chlorination by-products.

Acknowledgment

This study was financially supported by Natural Sciences and Engineering Research Council (NSERC) Canada Industrial Chair on Drinking Water Treatment at Ecole Polytechnique de Montreal, Canada. The assistance of Dr. Benoit Barbeau, Jacinthe Mailly, Julie Philibert, Marcelin Fosting, Yves Fontaine, Mireille Blais, Yan Wang and Lila Azouz is gratefully acknowledged.

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APPENDIX 5: Toxic cyanobacterial breakthrough and accumulation in a drinking water plant: A monitoring and treatment challenge

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Abstract:

The detection of cyanobacteria and their associated toxins has intensified in recent years in both drinking water sources and the raw water of drinking water treatment plants (DWTPs). The objectives of this study were to: 1) estimate the breakthrough and accumulation of toxic cyanobacteria in water, scums and sludge inside a DWTP, and 2) to determine whether chlorination can be an efficient barrier to the prevention of cyanotoxin breakthrough in drinking water. In a full scale DWTP, the fate of cyanobacteria and their associated toxins was studied after the addition of coagulant and powdered activated carbon, post clarification, within the clarifier sludge bed, after filtration and final chlorination. Elevated cyanobacterial cell numbers (4.7×10^6 cells/mL) and total microcystins concentrations (up to 10 mg/L) accumulated in the clarifiers of the treatment plant. Breakthrough of cells and toxins in filtered water was observed. Also, a total microcystins concentration of 2.47 mg/L was measured in chlorinated drinking water. Cyanobacterial cells and toxins from environmental bloom samples were more resistant to chlorination than results obtained using laboratory cultured cells and dissolved standard toxins.

Keyword: Cyanobacteria, Cyanotoxins, Treatment breakthrough, Drinking water

1. Introduction

Climate change and population growth contribute to increasing water temperature and nutrient loads and may intensify the proliferation of potentially toxic cyanobacteria in drinking water sources (Dale et al., 2006; Elliott et al., 2006; Jo¨hnk et al., 2008; Paul, 2008), even in temperate climates (Wiedner et al., 2007). Several cyanobacteria species are potent producers of a variety of toxins responsible for intermittent but repeated widespread poisoning of wild and domestic animals, fish from aquaculture, and humans (Carmichael et al., 2001). The increasing frequency and intensity of cyanobacterial proliferation leading to neurotoxin and hepatotoxin production is a universal problem (Chorus and Bartram, 1999; Svrcek and Smith, 2004; Merel et al., 2010). Reported human health effects of cyanotoxins include gastroenteritis, liver damage, neurotoxic effects and liver cancer. Microcystins (MCs) and cylindrospermopsin (CYN) are the most frequently identified toxins but toxins associated with bloom events have not always been identified (Svrcek and Smith, 2004; The Cooperative Research Centre for Water Quality and Treatment (Australia), 2007).

Several studies have documented the presence of toxic CB blooms in surface water and at water intakes of drinking water treatment plants (DWTPs) (Carmichael et al., 2001; Lahti et al., 2001; Svrcek and Smith, 2004; MDDEP, 2008; Merel et al., 2010; McQuaid et al., 2011). In a comprehensive national survey including 26 sampling sites over 24 months, maximum concentrations of w1000 mg/L of microcystin-LR (MC-LR) have been reported in French surface water (Agence Franc¸aise de Se´curite´ Sanitaire des Aliments (AFSSA) and Agence Franc¸aise de Se´curite´ Sanitaire de l'Environnement et du Travail (AFSSET), 2006). MCs have been detected in 33 American drinking water sources but MCs concentrations exceeded 1 mg/L in only 7% of samples (Haddix et al., 2007). Robert et al. (2005) monitored CB and cyanotoxins (Anatoxin, MC-LR, MCRR and MC-YR) every two weeks in the water intakes of six DWTPs located in southeast of Quebec (Canada), including a plant located on Missisquoi Bay on the Canadian side of the Lake Champlain 24 times over 3 years. MC-LR was found to be the dominant toxin present with maximum concentration of 3.5 mg/L in raw and 0.04 mg/L in treated water (Robert et al., 2005). The Missisquoi Bay is prone to cyanobacterial proliferations because of its high nutrient loadings (Simomeau, 2007). Using event-based sampling specifically targeting blooms, McQuaid et al. (2011) reported much higher values of cyanobcateria and MCs at this water intake with concentration of total intracellular and dissolved MCs up to 127 mg/L

MC-LR equivalents (MC-LR eq.). During the 2004 Wuxi drinking water crisis in Lake Taihu (China) maximum dissolved MCs concentrations of 35 mg/L were detected in the Meiliang Bay in Lake Taihu (Liu et al., 2011).

The impact of various treatment processes on dissolved cyanotoxins has been thoroughly reviewed and the limitations of various treatment processes are mostly established (Svrcek and Smith, 2004; Westrick, 2008; Lin et al., 2009; Merel et al., 2010; Zamyadi et al., 2010). However, cyanotoxins are most often cell-bound and it is essential to verify the fate of cyanobacteria cells and their associated toxins from real bloom events inside the DWTPs (Park et al., 1998; McQuaidet al., 2011). The impact of treatment of cyanobacterial cells is summarized in the Supplementary Data (SD) section Table SD-I1.

The physical removal and potential accumulation of cyanobacteria cells and their aggregates in treatment processes are affected by their size, charge, motility, morphology, and resistance to sheer stress and pressure, and these factors vary widely among species (Drikas et al., 2001; Dickens and Graham, 1995; Bernhardt and Clasen, 1991). Bacterial flocs of CB are smaller than 100 mm (American Water Works Association (AWWA), 2010). Pumping, coarse screening (>1mm), grit removal and forced air stripping are not efficient to remove cyanobacteria but may lead to cell damage. Micro-screening can be efficient depending on mesh size; a 35 mm screen can retain from 40 to 70% of phytoplankton and 10% or less of specific CB, including *Microcystis aeruginosa*, with no impact on dissolved toxins (Chorus and Bartram, 1999). The impact of cell surface and species-specific coagulant demand on the efficacy of clarification processes has been studied at various scales of experimentation and recently reviewed (Henderson et al., 2008). Reported efficiencies of cyanobacteria removal by coagulation/flocculation and sedimentation vary from 62% to 98.9% (Jiang et al., 1993; Vlaski et al., 1996; Jiang and Graham, 1998; Drikas et al., 2001) and are attributed to low cell density (Henderson et al., 2008). The accumulation of cyanobacteria and cell bound toxins in water treatment processes is expected and can cause toxin accumulation and subsequent modest toxin release into the treatment plant (Pietsch et al., 2002). Significant accumulation of cell bound MC-LR in gravity filters has been documented at pilot scale using native blooms dominated by Planktothrix rubescens (Pietsch et al., 2002; Schmidt et al., 2009). Rapid sludge removal from sedimentation basins is recommended to ensure that few additional dissolved toxins are released

from the sludge beds. Additionally, recycling of sludge drying beds should not be conducted until all toxins have degraded (European Commission, 2005).

Various oxidants including ozone, chlorine, potassium permanganate, chlorine dioxide have been used for the oxidation of cyanobacteria resulting in cell damage and oxidation of cell contents (SD section Table SD-I1). The susceptibility of cyanobacteria cells to oxidation is influenced by the species present, the physiological state of the cells, i.e. the growth phase (Pietsch et al., 2002), and the conditions of oxidation. It has been documented that during oxidation (e.g. chlorination) of a cyanobacterial suspension, the rate of toxin release is determined by the rate of the loss of membrane integrity (Daly et al., 2007; Zamyadi et al., 2010). The efficiency of the oxidative agent in released cyanotoxins oxidation depends on: the toxins present, the concentration of released toxins, the water quality and the contact time (Rodriguez et al., 2007; Ho et al., 2010; Zamyadi et al., 2010).

It must be noted that most studies on the elimination of cyanobacterial cells and toxins have been conducted at laboratory and pilot scale, allowing for the quantification of the impact of water quality and cyanobacterial speciation. Few research projects have confirmed the anticipated performances at pilot scale and full scale under typical plant operational conditions (Pietsch et al., 2002; Schmidt et al., 2002; Lin et al., 2009). Data available on the fate of cyanobacteria cells and their associated toxins from real bloom events inside the DWTPs are scarce, and preliminary evidence shows their potential breakthrough and accumulation during blooms (Lepisto et al., 1994; Suthaker and Drachenberg, 2007).

Elements of cyanotoxin management plans for drinking water plants include: (a) mandatory guidelines and standards for cyanotoxins in drinking water, (b) establishment of a plan to monitor cyanobacteria and their associated toxins, and (c) treatment methods for removing cyanotoxins (Chorus and Bartram, 1999; Newcombe, 2009). The recommended toxins alert levels are presented in the SD section Table SD-I2. The routine detection of relevant toxins for health and water treatment in source water raises several challenges: (1) toxin production is transitory in nature and difficult to predict; (2) the types of toxin(s) produced vary in time and space; (3) the relationship between the number of cyanobacteria present in water and the probability of the presence of cyanotoxins is complex; and (4) tedious and expensive analytical methods must be used to detect several types of toxins (Codd et al., 2005; Gregor et al., 2007). Based on

cyanobacterial cell enumeration and potential cyanotoxin concentration, monitoring strategies for drinking water (e.g. weekly counts and toxin analysis in DW supplies if 100,000 cells/mL are detected) are applied by different public health organizations (SI section Table SD-I3). In vivo measurement of cyanobacterial presence using high frequency monitoring probes can be used to monitor cyanobacteria in source waters (Ahn et al., 2007; Gregor et al., 2007; McQuaid et al., 2011). McQuaid et al. (2011) proposed a biomass alert level for in vivo cyanobacterial monitoring at the water intake of the DWTP situated in the Missisquoi Bay.

The overall objective of this study was to document the accumulation and fate of cyanobacterial cells and their associated toxins in a full scale DWTP coupled with online in vivo monitoring of cyanobacteria at the water intake. The purpose was to quantify the vulnerability of DWTPs to peaks of cyanotoxin concentrations resulting from breakthrough and accumulation of toxic cyanobacteria under realistic operational conditions. This demonstration will help define the scale of monitoring and treatment challenges. The specific objectives of this study were to 1) estimate the accumulation and breakthrough of toxic cyanobacteria in water, scums and sludge inside a DWTP, and to 2) study if pre- and/or postchlorination is an efficient barrier to prevent the breakthrough of cyanotoxins into drinking water.

2. Material and Methods

2.1. Water source and site description

The water intake of a the water treatment plant located on the Missisquoi Bay of Lake Champlain in southern Quebec (Canada) was monitored over three bloom seasons from June to October, 2008, 2009 and 2010. The body of water serves as a source of drinking water for the surrounding municipalities and as a recreational site for boating, fishing, and swimming. Characteristics of the treatment processes are presented in Table 1. The cyanobacteria bloom water samples for chlorination experiments were sourced from a bloom event in Missisquoi Bay in close proximity of the water intake. In this water the dissolved organic carbon (DOC) concentration was 5.4 mg/L. Prior to DOC analyses, samples were passed through pre-rinsed (1 L ultrapure water) 0.45 mm cellulose nitrate Supor_-450 membrane filters (PALL Life Sciences, USA). DOC measurements were made on a 5310C total organic carbon analyzer (Sievers Instruments Inc., USA).

Table 1. Characteristics of the treatment process used in the studied DWTP situated at Missisquoi Bay (the plant also uses Sodium hydroxide for pH adjustment).

Water Treatment Step	**Water quality& Chemical**	**August 21st, 2008**	**July 14th, 2009**	**July 29th, 2010**	**Specification**
Raw water	Turbidity (NTU)	19.8	15.9	22.9	-
	pH	8.0	7.6	8.6	-
	Temperature (^oC)	21.5	20.5	23.7	-
	DO[2] (%)	70.6	75.9	59.4	-
Polymer addition	Hydrex (Silicate) (mg/L)	0.07	0.09	Unknown	-
PAC[3] addition	PAC (mg/L)	9.3	7.1	Not registered	Wood based
Clarifier	-	-	-	-	Efficient clarifier depth: 4.90 m Maximum sludge bed depth: 2.95 m Hydraulic retention time: 55 minutes Solid retention time: 42 hours
Sludge tank for extraction	-	Age: 2 days	Age: less than one day	Unknown	-
Sand-Anthracite Filter	-	-	-	-	Sand effective size: 0.5 mm Sand uniformity coefficient: 1.6 Sans thickness: 15cm Anthracite effective size: 1.0 mm Anthracite uniformity coefficient: 1.7 Anthracite thickness: 61cm
Post-oxidation	Chlorine initial dose (mg/L)	1.2	2.0	Unknown	Minimum contact time 4h
	Chlorine residual in distributed water (mg/L)	0.71	0.84	0.80	-
	Estimative CT[4] (mg.min/L)	117.6	278.4	108.4	-

[1] Dissolved organic carbon

[2] Dissolved oxygen

[3] Powdered activated carbon

[4] Contact Time: exposure to chlorine

2.2. In vivo phycocyanin monitoring

An online YSI 6600 V2-4 water-qualitymulti-probe (YSI, Yellow Springs, Ohio, USA) fitted with an in vivo phycocyanin fluorescence (YSI6131 Blue Green Algae) self cleaning "wiped" probe was used in this study (YSI Incorporated, 2006; Zamyadi, 2011). The phycocyanin probewas installed inside theDWTP at the raw water intake prior to any treatment and measurements recorded every 30min. The phycocyanin probe excites the cyanobacterial phycocyanin at 590 nm (with a pass-band of 565–605 nm) and measures the pigment's emission at 660 nm. The probe detects light emitted in the range of 640–680 nm. The probe raw readings are presented in ratio fluorescent units (RFU). The probe resolution, detection limit and limit of quantification is 0.1 RFU over its range of 0–100 RFU, 0.2 RFU) and 0.7 RFU, respectively (McQuaid et al. 2011).

2.3. Sampling procedure

Weekly field visits were conducted on a fixed day to collect probe records and water samples were taken from the water intake only for microscopic taxonomy and toxin analysis. If RFU augmentations were observed in the probe readings within 24 hours prior to the sampling day or the presence of cyanobacteria was detected inside the DWTP, triplicate water samples were taken across the treatment processes from raw to treated water: raw water from the pipeline conducting raw water to the treatment facility prior to any treatment; surface of the reactive addition and flash mix tank; scum at the surface of the clarifier/sedimentation tank; sludge from the sludge bed of the clarifier using the sampling valve; concentrated sludge in the concentration cone using the sampling valve; scum over the filter; and treated water at the outlet of the reservoir (complete Cl2 contact time). Sampling taps were fully flushed before taking samples. Samples for taxonomic counts were preserved using Lugol's iodine and samples for total toxins analysis were stored in -25℃.

2.4. Field samples pre-treatment

Cells from all field samples were submitted to three freeze/thaw cycles before any analysis for total toxins. In 2010, field samples (5mL) were vortexed in conical centrifuge tubes (BD Falcon, 50mL, Fisher Scientific) at 3000 rpm for 2 minutes, treated with internal standard (25µL of 100µg/L nodularin (in methanol, providing final nodularin concentration of 500ng/L) and passed through syringe filter (10mL polypropylene syringes and 25mm diameter Whatman GD/X GMF,

0.45µm pore size filters) then divided into 3 separate 2mL amber autosampler vials. For this set of samples (an initial first test of the method with real samples) extracts were first analyzed as described. If concentrations exceeded the highest concentration in the calibration standard curve, subsequent dilutions were completed with appropriate addition of internal standard before analysis.

2.5. Chlorination experiments

Liquid chlorine stock solution was purchased from Fisher (USA). Chlorine stock and chlorine residual concentrations were determined using the DPD-FAS titration method described in Standard Methods (American Public Health Association (APHA) 1998). Typical chlorine stock solutions concentration is 50,000 mg/L as free chlorine. The experimental plan included chlorination of a sample of cyanobacteria bloom from Missisquoi Bay. A chlorine decay experiment was conducted prior to each experiment. For chlorine decay experiments, water samples were dosed with the desired chlorine concentrations (2 and 5 mg/L) and triplicate samples taken at specified contact times (0, 1, 2, 5, 10, 15, 30 and 60 min) for free chlorine residual determinations using the DPD-FAS titration method. Cyanobacteria cells exposure to chlorine is in CT (Contact Time), the residual concentration of the chlorine multiplied by time (Ho et al., 2006). The CT value is calculated by determining the area under a graph of chlorine concentration versus time. All chlorination experiments were conducted in triplicate in brown amber bottles at room temperature (20 ±2 ℃).

Environmental cyanobacterial bloom samples were taken from Missisquoi Bay (5.7×10^5 (±4%) cells/mL) where the speciation of the cyanobacterial population was conducted. Chlorine was added from the chlorine stock solution (2 and 5mg/L). Samples were quenched at specified contact times with sodium thiosulphate at a stoichiometric ratio specified in Standard Methods (American Public Health Association (APHA), 1998). Subsamples of the quenched samples were taken for DOC measurement. To determine cell lysis total cell microscopic counts were conducted in triplicate on 2-mL samples after preservation in Lugol's iodine (Lund et al., 1958; Wetzel, 2000). Remaining quenched water samples were divided in two subsamples of 250 mL: the first subsample was immediately filtered through 0.45 µm cellulose filter (Whatman, UK) to remove cellular material, and then analyzed for extracellular toxins. The second subsample was subjected to three freeze/thaw cycles before filtration over 0.45 µm filter to analyze for total

toxins (both the cell-bound and extracellular). For all experiments the extracellular concentrations of toxins analysis were always below their total concentration. The difference between total and extracellular levels of toxins was estimated as the cell-bound concentration.

2.6. Chemicals and reagents

Standard solutions of MC-LR, cylindrospermopsin, MC-RR, nodularin, dm MC-LR were purchased from National Research Council (Halifax, NS). Anatoxin-a was purchased as anatoxin-a fumarate from Cedarlane (Burlington, ON) and MC-LF, MC-LW, MC-LY, MC-YR were all purchased from DHI (Hørsholm, Denmark). Individual toxins were diluted to 1 mg/L with HPLC-grade MeOH and kept in amber vials at -20°C. Working solutions with all toxins for external calibration (10, 50, 100, 250, 500, 1000, 5000, 10000ng/L each with 500ng/L internal standard) were prepared via appropriate dilutions with HPLC grade H_2O and were kept at 4°C prior to and during analysis. All chemicals and reagents were analytical grade from various suppliers.

2.7. Analysis of toxins

For the 2008, 2009 and chlorination experiment samples MC-LR analyses were conducted using an Abraxis Microcystin ELISA Plate (an enzyme-linked immunosorbent assay – Abraxis LLC, Pennsylvania, USA). The analyses of MC-LR concentrations were measured as mg/L of MC-LR eq.. The accuracy of the Plate was validated using standard toxin materials and the average standard deviation of triplicate measurements was 3% (SD section Table SD-I4).

The 2010 toxin analysis was carried out via online solid phase extraction-liquid chromatography coupled to tandem mass spectrometry (online SPE-LC-MS/MS) consisting of a CTC Autosampler (from Thermo by Leap Technologies), a Thermo Fisher (Waltham, MA, USA) HPLC system (with Accela 1250/analytical and 600/loading pumps), one 6- and one 10-port switching valve (VICI), a HotPocket column heater (Thermo Fisher) and a TSQ Quantum Ultra AM Mass Spectrometer (Thermo Fisher) with an Ion Max atmospheric pressure ionization (API) source (Thermo Fisher). The method, adapted from published multi-toxin (Dell'Aversano et al. 2004; Spoof et al. 2010; Oehrle et al. 2010) and online SPE-LC-MS/MS methods (Stoob et al. 2005; Segura et al. 2007), allowed for determination of ng/L toxin concentrations in 1 mL samples without arduous pre-concentration. The runtime was 6.8 minutes/sample with recoveries ranging from 97-109%. Hypersil GOLD (Thermo Fisher, Waltham MA USA) LC columns were

employed in online extraction (12µm, 20 × 2.1mm) and analyte separation (1.9µm, 50 x 2.1mm) with a stepped gradient of formic acid and acetonitrile as the mobile phase. MC-LR, MC-YR, MC-LY, MCLF, MC-RR, MC-LW, 3-desmethylMC-LR (dm-MC-LR), and CYN toxins were analyzed by this method.

3. Results and discussion

3.1. Breakthrough and accumulation of toxic cyanobacteria inside a DWTP

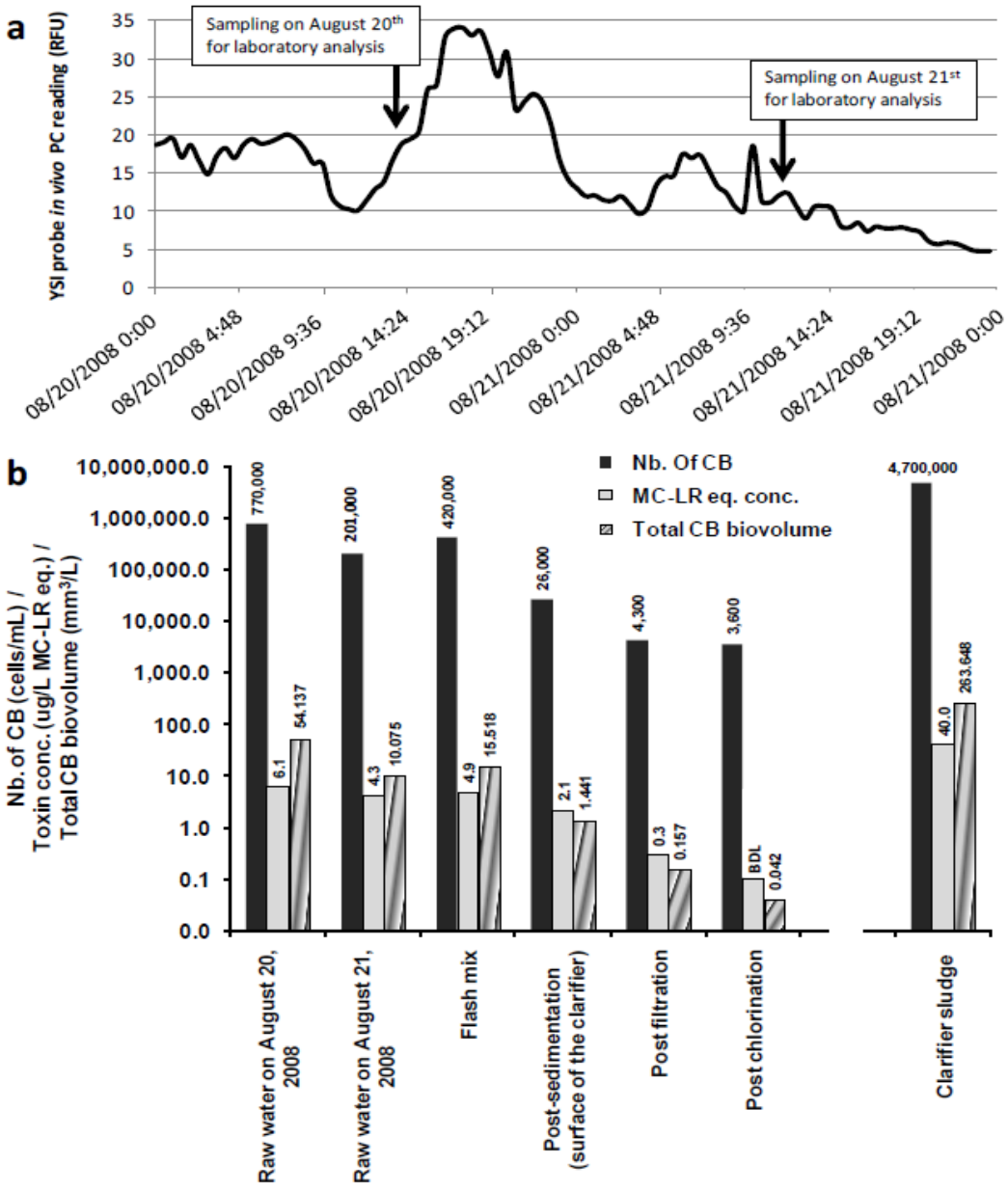

Fig. 1 Breakthrough and accumulation of cyanobacteria cells in the studied DWTP: (a) in vivo intensive monitoring at the raw water (arrow points sampling time for laboratory analysis) with the x axes in month/day/year hour:minute, and (b) cell counts, toxin analysis and total cyanobacteria biovolume estimation in raw water (August 20th and 21st, 2008) and after different treatment processes (August 21st, 2008) (BDL: below detection limit).

On the afternoon of August 20th, 2008 a notable increase of cyanobacteria was recorded by the in vivo phycocyanin probe (Fig. 1a). Raw water at the water intake of the DWTP contained 7.70×10^5 cells/mL of cyanobacteria and 6.1 µg/L of MC-LR eq.. *Microcystis* sp., *Anabaena* sp., *Pseudanabaena* sp., *Aphanothece* sp., *Aphanocapsa* sp., *Glaucospira* sp., *Planktolyngbya* sp. And *Aphanizomenon* sp. were identified in this sample with M. *aeruginosa* being the dominant species representing 61% and *Anabaena* representing 33% of the total cyanobacterial biovolume. The flux of cyanobacterial cells into the DWTP decreased on August 21st, 2008 as shown by probe readings and a cyanobacteria count of 2.01×10^5 cells/mL with *M. aeruginosa* species representing 74.5% and *Anabaena* sp.. Approximately 16% of the total cyanobacterial biovolume (Fig. 1b). McQuaid et al. (2011) proposed an alert level of 2.4 RFU (equivalent of 1mm3/L total cyanobacterial biovolume and maximum potential of 13 mg/L MC-LR) for YSI in vivo phycocyanin probe measurements at this water source. For these two days, the in vivo fluorescence measurements in raw water exceeded the proposed alert level while total cell-bound actual dissolved toxins remained much lower than the calculated maximum production potential.

During both days, the cumulative flux of coagulated cyanobacterial cells to the clarifier resulted in multi-fold accumulation (4.70×10^6 cell/mL) of toxic cyanobacteria corresponding to a significant mass of 4.92 g MC-LR eq. present in the total volume of the sludge bed of the clarifier (Fig. 1b). As the solids retention time is estimated at about 42 h (2% purge) the accumulation of cells in the sludge should reflect the composition and toxin content of the incoming cyanobacteria, unless selective removal of certain species occurs. Interestingly, the cell speciation in the sludge bed did remain similar with a dominance of *Microcystis* sp. (82% of biovolume) and *Anabaena* sp. (14% of biovolume) and a relatively constant toxin cell content of 0.08 pg MC-LR eq./cell. The sludge blanket clarifier removed 86% of the total cyanobacterial cell number present in the raw water and reduced total toxins to 2.1 mg/L of MC-LR eq.. Although the same cyanobacteria species were identified in post-clarifier water, a shift in the most abundant species was observed with a dominance of *Aphanizomenon* sp. (1.2×10^4 cells/mL, 76.3% of biovolume) and *Aphanothece* sp. (4.7×10^3 cells/mL, 2.4% of biovolume). Table 2 shows that clarifier efficiency in removal of cyanobacteria total biovolume is species dependent. Dual media filtration further reduced the cyanobacteria cells to 4.3×10^3 cells/mL (0.157mm3/L) with *Anabaena flos-aquae* and *Aphanothece minutissima* remaining the most abundant species. However, in filtered water, small concentrations of 7.3×10^2 cells/mL of *Microcystis* sp. still

represented 32% of the total biovolume because of its elevated cell size. Post chlorination with a minimum CT of 117 mg min/L was not efficient in lysing the cyanobacteria cells with a modest 16% reduction in the remaining cell numbers and elimination of MCs below the detection limit (0.1 µg/L). However the average cell biovolume was considerably reduced from 57 to 12 μm^3/cell as a result of the preferential lysis of the remaining *Anabaena* sp. and *Microcystis* sp.

Table 2. Cumulative percentage of trapped cell after each treatment process with their estimated total biovolume from microscopic taxonomy

Treatment process	**August 21st, 2008**				**July 14th, 2009**			
	APHAZ+APHAC*		**MIC+ANA****		**APHAZ+APHAC**		**MIC+ANA**	
	Biovolume (mm^3/L)	% of accumulative removal	Biovolume (mm^3/L)	% of accumulative removal	Biovolume (mm^3/L)	% of accumulative removal	Biovolume (mm^3/L)	% of accumulative removal
Post-clarifier	1.103	-56.0	0.321	96.5	3.228	-931	2.401	-51.4
Post-filtration	0.101	85.0	0.053	99.4	0.229	26.8	0.064	96
Post-chlorination	0.020	97.9	0.015	99.7	0.001	99.3	0.000	100

* *Aphanizomenon* sp. (APHAZ) + *Aphanothece* sp. (APHAC)

** *Microcystis* sp. (MIC) + *Anabaena* sp. (ANA)

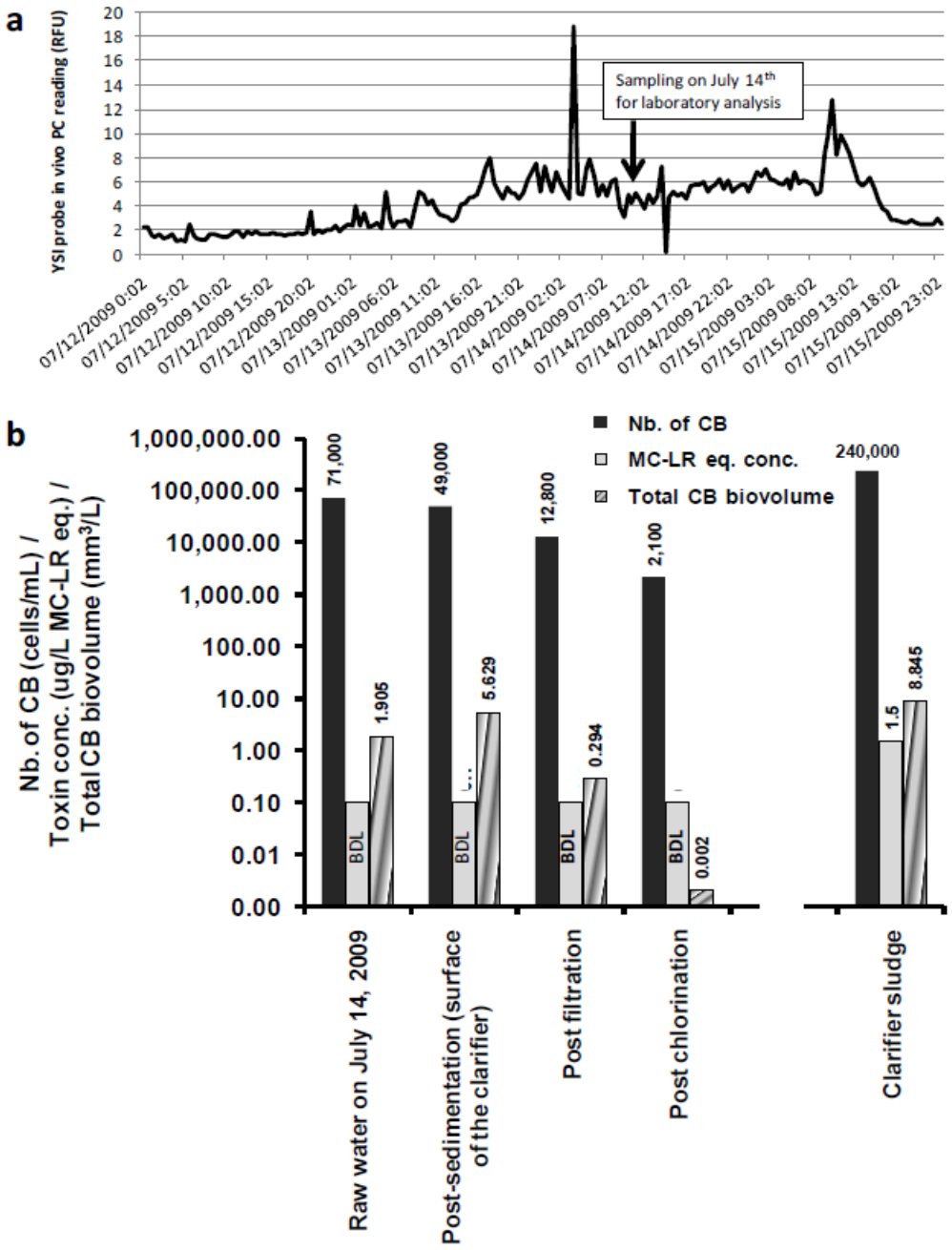

Fig.2 Breakthrough and accumulation of cyanobacteria (CB) cells in the studied DWTP on July 2009: (a) in vivo intensive monitoring at the raw water (arrow points sampling time for laboratory analysis) with the x axes in month/day/year hour:minute, and (b) cell counts, toxin analysis and total cyanobacteria biovolume estimation after different treatment processes on July 14th, 2009.

Fig. 2a presents the increasing flux of cyanobacteria cells into the DWTP from early morning hours of July 12th, 2009. The in vivo phycocyanin probe readings exceeded the proposed alert level of 2.4 RFU on July 13th, 2009 (Fig. 2a). On July 14th 2009, the dominant cyanobacteria in the raw water were *M. aeruginosa* (2.5×10^4 cells/mL, $1.6mm^3/L$) but it also included *Aphanothece clathrata brevis* (2.4×10^4 cells/mL, $0.013mm^3/L$), *Aphanocapsa delicatissima* (1.8×10^4 cells/mL, $0.007mm^3/L$), and *Aphanizomenon flos-aquae* (4.0×10^3 cells/ mL, $0.300mm^3/L$) (Fig. 2b). The toxin production of these cyanobacteria cells was below the detection limit.

Similarly in 2008 (Fig. 1b) the cumulative flux of coagulated and flocculated cyanobacteria cells to the clarifier caused major cyanobacteria cells accumulation in the sludge bed of the clarifier (2.4×10^5 cells/mL e Fig. 2b). *M. aeruginosa* was the dominant species (84.51% of the total biovolume) in the settled cyanobacteria cell (in sludge) followed by *A. flos-aquae* (15.48% of the total biovolume) and A. clathrata brevis (0.01% of the total biovolume). The MCs concentration in the sludge bed was 1.5 MC-LR eq. and *M. aeruginosa* was the dominant species over the clarifier followed by *A. flos-aquae* and *A. clathrata brevis*. However, the total biovolume of small-sized *A. clathrata brevis* ($0.5\ \mu m^3$/cell) over the clarifier was approximately two times greater than these cells total biovolume in the settled sludge. This outcome is in complete coherence with the -931% removal of the total biovolume of this species after the clarifier (Table 2). A. clathrata brevis was the dominant species in post-filtration water when considering total cell numbers. However, 76.7% of the total biovolume in filtered water consisted of A. flos-aquae, followed by *M. aeruginosa* (21.9%) and *A. clathrata brevis* (1.4%). These results highlight the importance of presenting the results in a scale unit that has a better coherence with the reality (i.e. biovolume rather than cell numbers). *A. clathrata brevis* was the only species detected in the chlorinated water (Fig. 2b).

These results are in agreement with the cyanobacteria removal efficiencies (62e98.9%) of coagulation/flocculation and sedimentation processes published previously (Jiang et al., 1993; Vlaski et al., 1996; Jiang and Graham, 1998; Drikas et al., 2001). Coagulation can be optimized with an online system measuring the zeta potential (Bernhardt and Schell, 1993; Bernhardt and Clasen, 1994; Lai et al., 2002). Our observations provide novel data on cyanobacteria cell breakthrough in the dual media sandeanthracite filter used in this DWTP (Figs. 1b and 2b). Cyanobacteria cells were also detected in chlorinated water for both years as previously reported

for *Anabaena circinalis* (Zamyadi et al., 2010). However, cell integrity analysis showed that all of those cells were metabolically inactivated (Zamyadi et al., 2010).

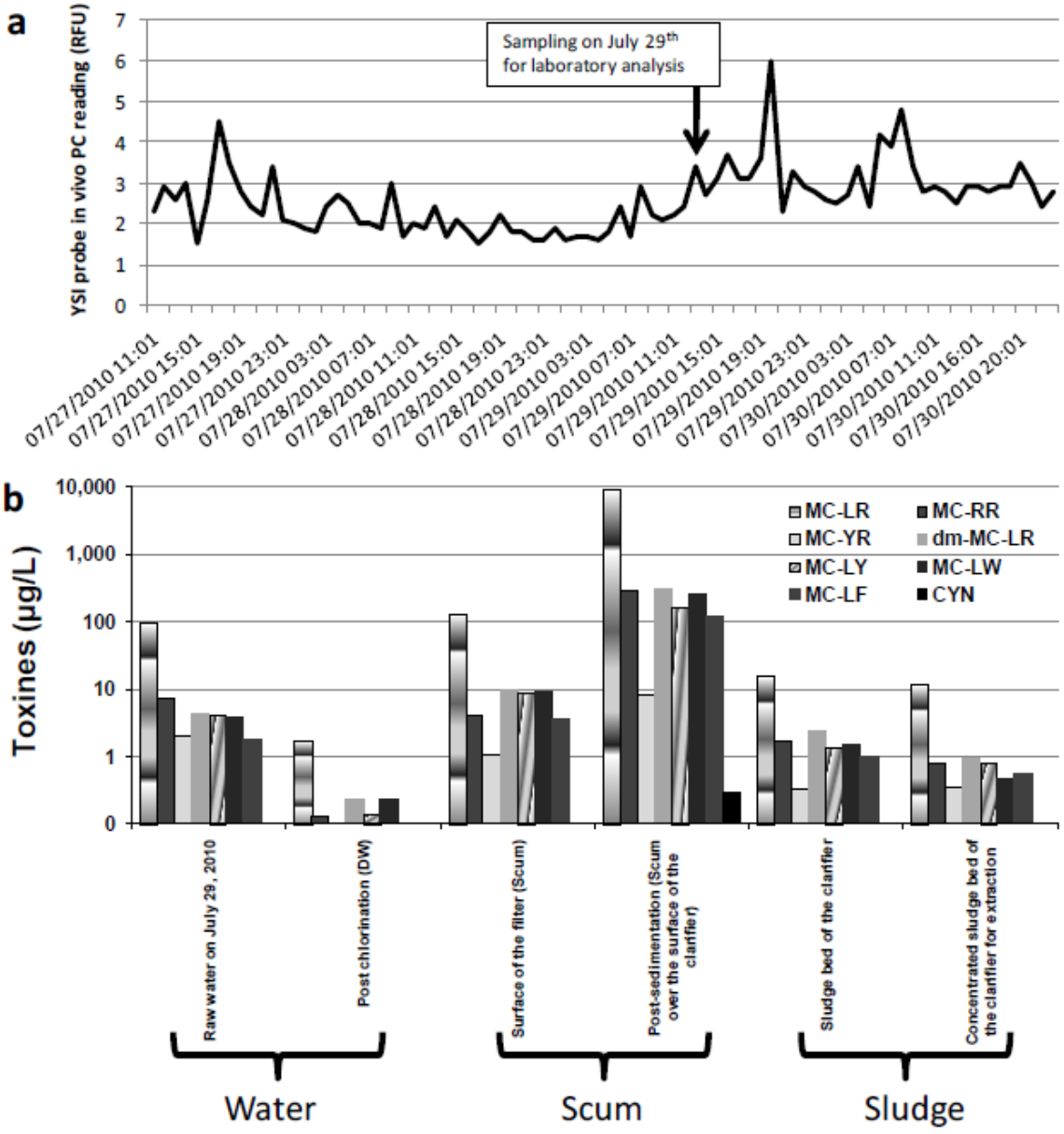

Fig.3 Breakthrough and accumulation of cyanobacteria (CB) cells on July 2010: (a) in vivo intensive monitoring at the raw water (arrow points sampling time for laboratory analysis) with the x axes in month/day/year hour:minute, and (b) cell counts and toxin analysis after different treatment processes on July 29th, 2010.

In 2008 and 2009 the breakthrough and accumulation of cyanobacteria cells in the DWTP was caused by an influx of high cyanobacteria cell numbers into the facility as shown by parallel high in vivo peaks (Figs. 1 and 2). However, toxin production did not in any way follow the same patterns. In 2010, even though *in vivo* cyanobacteria cell measurements were lower than the previous years (Fig. 3a) the unit cell toxin production was higher when compared to previous years (Fig. 3b). The proportion of different cyanotoxins present in water samples are also presented in Fig. 3b. MC-LR remained the dominant toxin with observed traces of CYN b of great interest (Fig. 3b).

The total MCs concentration at the water intake of the DWTP reached 118.70 mg/L. Powdered activated carbon (PAC) has been shown to be efficient to remove dissolved MCs and CYN (Ho et al., 2011). Significant removal of MCs has been observed during the pre-treatment stage in DWTPs using PAC addition to the raw water (American Water Works Association (AWWA), 2010). However, the PAC dose, the PAC type, the water quality and the type of cyanotoxin influence its efficacy. PAC efficiencies can vary from 20 to 80% toxin removal for a dose of approximately 10 mg/L of PAC (Ho et al., 2011). In the studied DWTP even though PAC was added to the raw water, high toxin concentrations were detected after the addition of PAC. PAC does not remove intact cells and cell-bound toxins.

A green scum was observed over the clarifier and 10,300 mg/ L of MCs and 0.29 of mg/L of CYN were detected in the scum sample (Fig. 3b). The presence of a green scum at the surface of the clarifier confirms the breakthrough from the sludge bed and their co-current movement, accumulation and potential development over the settled water collecting pipes. Table 2 shows that this phenomenon had also occurred in 2008 and 2009. The negative removal of total biovolume of cyanobacteria cells in clarified water (Table 2) is due to release of cyanobacteria cells from sludge. Poly-aluminum chloride is used as coagulant in this DWTP. Recent studies have observed coagulation inhibition caused by surface-related organic matter and proteins derived from M. aeruginosa while using Poly-aluminum chloride (Takaara et al., 2007, 2010; Sano et al., 2011). Furthermore, during the cyanobacterial proliferation period, the flocs are light. It is therefore recommended that coagulant aids, e.g. anion polymers, be used for the flocculation process (Bernhardt and Clasen, 1994). In certain cases, the use of a flocculating agent containing cationic (Agence Franc¸aise de Se´curite´ Sanitaire des Aliments (AFSSA) and Agence Franc¸aise de Se´curite´ Sanitaire de l'Environnement et du Travail (AFSSET), 2006) or anionic

(Bernhardt and Clasen, 1993) polymers would allow a better elimination of the cyanobacteria. Furthermore, aluminum sulfate can be a more efficient flocculating agent than iron salts (Pietsch et al., 2002). Significant cyanotoxin release during flocculation/filtration has been reported (Pietsch et al., 2002). Hydraulic stress (turbulence) caused by flocculation/filtration and pressure gradients in pipes and filters have a destabilizing effect on the integrity of the cyanobacterial cells. However, the stability of cyanobacterial cells varies according to their growth phase, thus, depending on their growth phase; flocculation/filtration may lead to the release of toxins (Pietsch et al., 2002). Pietsch et al. (2002) showed that cells captured in the clarifiers can release all cyanotoxins within the first 48 h in clarifiers with solid retention time of over 48 h. The estimated sludge retention time in the clarifiers in this study was approximately 42 h (Table 1). Cyanobacteria lysis retained on the filters can occur after 24e48 h of retention and good management of the filters maintenance process is paramount (Lepisto et al., 1994; Chorus and Bartram, 1999).

Total MCs concentration in chlorinated drinking water reached 2.47 μg/L (Fig. 3b) exceeding the recommended MCs alert levels for drinking water (SD section Table SD-I2). MC-LR was the dominant analog with the highest concentration (1.74 μg/L). Results from laboratory scale chlorination experiments are contradictory. While chlorination of dissolved MCs in ultrapure and natural water was efficient (Rodriguez et al., 2007; Ho et al., 2010), Daly et al. (2007) observed 14.1e94.1% removal of total MCs produced by M. aeruginosa cells. Thus, 2010 toxins monitoring demonstrates that it is essential to verify the chlorination efficiency in the presence of high concentrations of toxins and cellular materials, and questions the strength of chlorination as a barrier as predicted by laboratory kinetic oxidation trials.

A total MCs concentration of 15.5 μg/L was measured in the concentrated sludge of the clarifier (in the sludge concentrator for extraction). Toxic cyanobacteria management in the DWTP must also consider the permanent elimination and/or reuse of the sludge. Sludge treatment needs particular attention as intracellular toxins might be released during sludge handling and supernatant recycling could increase the loads of toxins for treatment.

The recent published guidelines recommend cyanobacteria biweekly monitoring at alert level 3 (65,000 cells/mL) (Newcombe, 2009; Newcombe et al., 2010). Our observations suggest that low frequency sampling may lead to the underestimation of significant peak of toxic cyanobacteria

influxes into the DWTP. Our results also suggest that lower cyanobacteria influx accumulating over a long period in the clarifier could also lead to significant accumulation and potential release. Figs. 1b and 2b show that the breakthrough of cyanobacteria cells through post-chlorination is possible under these conditions.

3.1. Chlorination of Missisquoi Bay cyanobacteria bloom water sample

3.2.1 Impact of cyanobacteria cells and cell bound toxins on chlorine delay

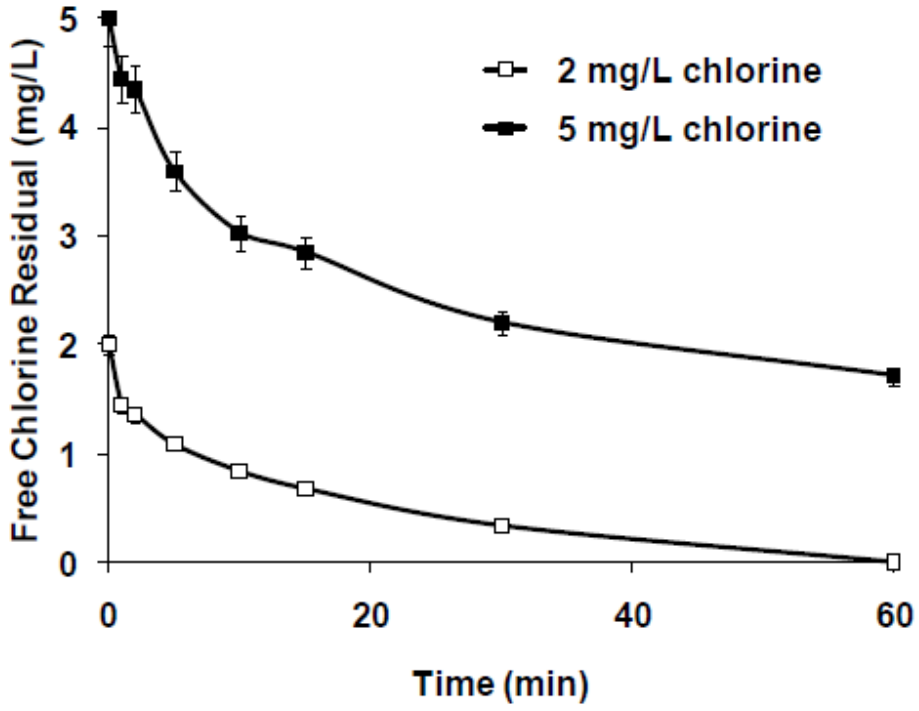

Fig.4 Chlorine decay in Missisquoi Bay bloom water samples with 5.7×10^5 (±4%) cells/mL at ambient pH (pH before chlorination was 7.8; pH augmented to 8.1 and 8.3 after chlorination with 2 and 5mg/L Cl_2 respectively, and remained constant for the rest of the experiment).

Free chlorine decay and microscopic cell counts were conducted using the Missisquoi Bay cyanobacteria bloom samples at cell number of approximately 5.7×10^5 (±4%) cells/mL, at two chlorine doses, 2 and 5mg/L as Cl_2 (Fig. 4). The standard deviation value of these cell counts (triplicate count per sample) fits within the confidence limit of cell enumeration methods (Lund et al., 1958; Laslett et al., 1997) (data not shown). CT values were calculated from chlorine decay data by fitting a two pathway integration model to a two phase decay curve: fast (<2 min) and

slow (Sohn et al., 2004). The resulting first order rate constants for 2 and 5 mg/L of chlorine decay are 1.1×10^{-3} ($r^2 = 0.92$) and 1.0×10^{-3} ($r^2 = 0.97$), respectively.

3.2.2. Effect of chlorination on cyanobacteria cells and toxin release

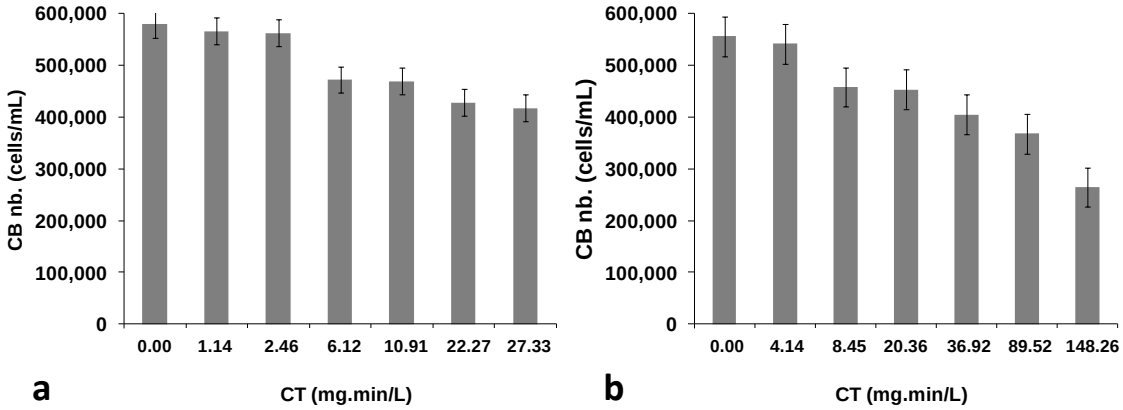

Fig. 5 Cell numbers after chlorination of Missisquoi Bay bloom water samples using (a) 2 mg/L Cl_2, and (b) 5 mg/L Cl_2

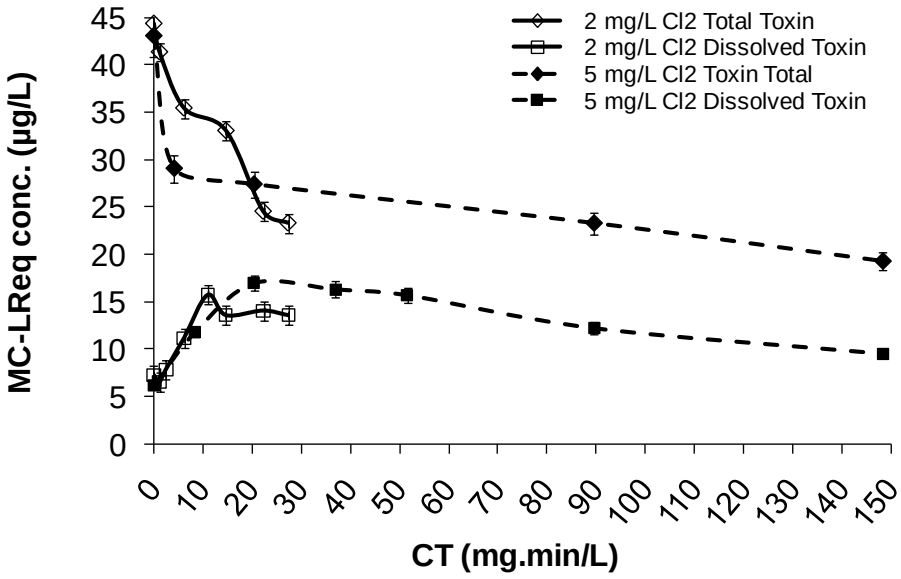

Fig.6 Toxins release and oxidation after chlorination of Missisquoi Bay bloom water samples using 2 and 5 mg/L Cl_2

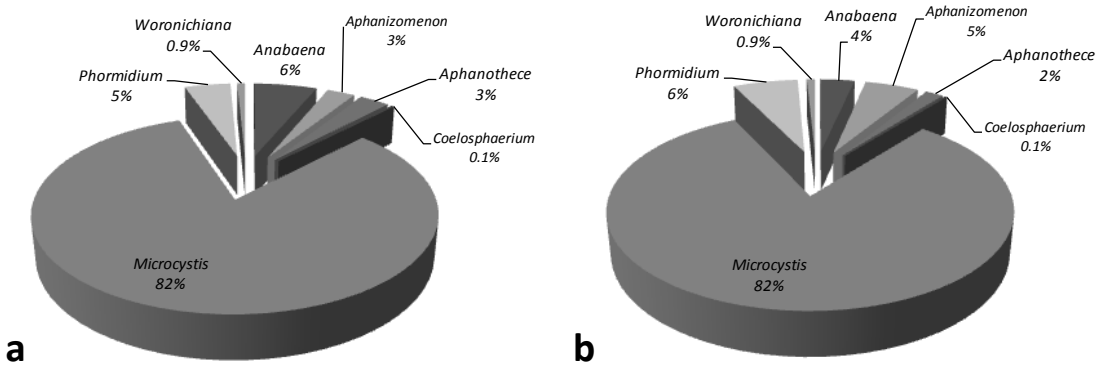

Fig.7 Proportion of seven cyanobacteria (CB) species out of total cyanobacteria cell in Missisquoi Bay bloom sample (a) before chlorination (5.7×105 (±4%) cells/mL), and (b) after chlorination using a CT of 148 mg.min/L (2.7×105 (±4%) cells/mL – 53% reduction of total cell numbers)

The response of a cyanobacteria bloom sample from Missisquoi Bay of approximately 5.7×10^5 (±4%) cells/mL to chlorine doses of 2 and 5 mg/L at ambient pH (8.3) was evaluated. For these bloom samples, 28% to 53% reductions of total number of cells, corresponding to full cell lysis, were observed at the end of the chlorination period for both chlorine doses (Fig. 5). However it is not possible to categorize the remaining cells as damaged membrane or metabolically inactive cells as cell-bound toxin are still observed even after the highest CT (148.26 mg.min/L) (Fig. 6). In previous studies (Daly et al. 2007; Lin et al. 2009; Zamyadi et al. 2010), fast release of cell-bound compounds have been observed when pre-chlorinating (with low chlorine doses, 1-5 mg/L Cl_2) natural water loaded with *Anabaena* sp. and *Microcystis* sp. However this study presents the first set of experiments using natural bloom samples incorporating seven different cyanobacteria species present but dominated by *Microcystis* sp (Fig. 7). Furthermore, after the chlorination with a CT of 148 mg.min/L and a 53% reduction in total cell number the proportion of cyanobacteria species with regards to total number of cells present was almost the same as before chlorination with slight declines in *Anabaena* sp. and *Aphanothece* sp (Fig. 7). DOC variations before and after chlorination were <0.2 mg/L. Similar results were observed in chlorination of natural bloom samples in a recent study (Zamyadi et al., in press).

It is possible to assume a first-order reaction with regards to chlorine reaction and the reduction of total cell numbers (Daly et al. 2007; Lin et al. 2009). Thus the reaction between chlorine and

the cells is assumed to be of second order and the rate at which cell lysis occurs (k_{lysis}) is evaluated using equation 1: .

$$Ln\left(\frac{[N_{CT}]}{[N_0]}\right) = -k_{lysis} \times CT \qquad \text{eq. 1}$$

where CT equals the chlorine exposure; N_{CT} equals the number of lysed cells after a given chlorine exposure; N_0 equals the number of cells at CT=0; and k_{lysis} equals the rate at which cell oxidation occurs (Zamyadi et al. 2010). The evaluated rates for cell reaction with chlorine from this study are in disagreement with the results of previous studies (Table 3). The vulnerability of the cells of the CB species to chlorination is influenced by the physiological state of the cells, cell densities, water matrix and the chlorination conditions (Hart et al. 1998; Pietsch et al. 2002; Lin et al. 2009). Howerer, the optimization of the chlorination process is limited by the formation of disinfection byproducts mainly due to background water quality (Zamyadi et al., in press).

Table 3. Cyanobacteria (CB) k_{lysis} ($M^{-1}s^{-1}$) derived from this study compared to published results (Daly et al. 2007; Lin et al. 2009; Ding et al. 2010; Zamyadi et al. 2010). Comments in parenthesis describe the origin of the CB cells (from laboratory cultures or environmental bloom samples).

This Study	2 mg/L Cl_2	5 mg/L Cl_2	Published papers	Daly et al. 2007	Lin et al. 2009	Zamyadi et al. 2010	Ding et al. 2010
CB species	Ambient pH (8.3)		CB species	pH 6.8-7.6	pH 8.3-8.6	pH 6.8-8	pH 7.6
Missisquoi Bay bloom sample with 7 species present	15.97	3.78	*A. circinalis*		1400-3400 (culture)	1400-1600	-
			M. aeruginosa	670±77	790-1100 (culture), 70-590 (bloom)	-	55.9

4. Conclusion

Sampling once or twice a week on fixed day fails to spot the highly temporal variability of cyanobacteria bloom presence at the water intake of the DWTP. Non-event based sampling causes an underestimation of the transitory cyanobacteria bloom events and the accumulation and breakthrough of toxic cyanobacteria in the DWTP. A monitoring strategy including intensive in vivo fluorescence measurements and event-based sampling would help to prevent the documented event of 2008-2010. DWTP operators should consider the details of the operational facilities before interpreting laboratory derived data for real environmental conditions. This paper provides the novel information on accumulation of potentially toxic cyanobacteria cells inside DWTPs, particularly the clarification and filtration processes. Future systematic studies of these phenomena in full scale operational DWTPs are required to establish the key factors for cyanobacteria accumulation and removal, and prepare efficient management plans.

Furthermore, our findings demonstrate that breakthrough of cyanotoxins at concentration exceeding the health-based exposure alert levels can occur, even for toxins considered to be readily oxidized by chlorine. Finally, the extrapolation of laboratory scale chlorination experiments based on dissolved toxins and cultured cyanobacteria species to the operational conditions requires further investigation.

Acknowledgment

This study was financially supported by Natural Sciences and Engineering Research Council of Canada (NSERC) Industrial Chair on Drinking Water at École Polytechnique de Montréal. The authors acknowledge the support of the operators at the MB DWTP, Yves Fontaine, Julie Philibert, Mouhamed Ndong, and Serge Paquet for their technical help.

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